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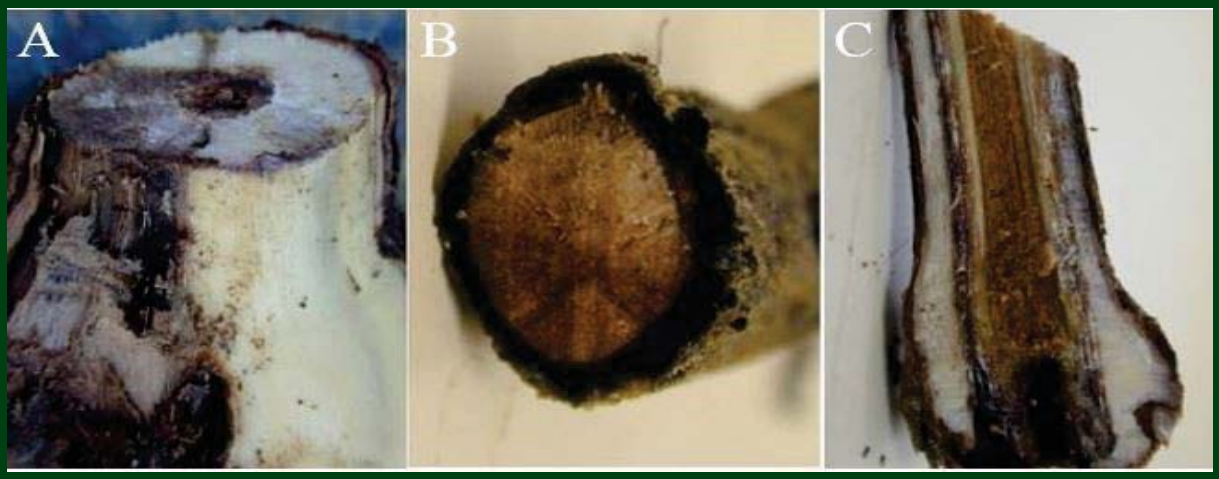

- The cross-section of rootstock infected by black foot disease (A), cross-section of the root (B) and longitudinal-section of a young grapevine infected with *Cylindrocarpon* spp. (C) [adapted with the permission from Halleen et al. (2007)].

Highlights

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REVIEW PAPER

Role of Grapevine Rootstocks in Mitigating Environmental Stresses: A review

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دور أصول العنب في تخفيف الضغوط البيئية: مراجعة

محمد ممتاز خان ١ *، محمد طاهر أكرم ٢ ، راشد وسيم خان قادري ٢، راشد اليحيائي١

ABSTRACT. Viticulture is one of the most important crop industries in the world and its cultivation is on the upward trend globally. Global water and soil resources continued to decline sharply and rampant extreme weather conditions are becoming a serious threat to sustainable agriculture and food security. Further, the changes in climatic conditions are increasingly becoming favorable for rearing certain harmful biotic organisms, which are hostile to sustained grape cultivation. The environmental changes have shown a projected negative impact on viticulture by increased biotic and abiotic stresses. Range of strategies can be employed to mitigate such scenarios, however, integration of rootstocks to combat such challenges is a sustainable nature. Grape rootstocks have exhibited their role in mitigating the problems raised due to a variety of environmental stresses. For example, certain Vitis species are used as rootstock against phylloxera and other harmful pests of grapes. Similarly, there are certain rootstocks developed which have their tolerance against salinity, drought, cold, and iron chlorosis. With ever-changing environmental conditions, it is not essential that one rootstock perfors better at a specific place may perform well in another place. This article reviewed several grape rootstocks which have their specific resistance or tolerance features against a variety of stresses, including pests, disease, salinity, and drought. Consistent endeavors in grapevine rootstock improvement and utilization are critical for the sustainability of the grape industry, in particular during the ever-increasing environmental stresses.

KEYWORDS: Drought; Phyloxera; Salinity, Rootstocks; Viticulture; Stress.

المستخلص:تعتبر زراعة الكروم واحدة من أهم الصناعات المحصولية في العالم وزراعتها في الاتجاه التصاعدي على مستوى العالم. واصلت موارد المياه والتربة العالمية انخفاضها الحاد وانتشار الأحوال الجوية القاسية ، وأصبحت تشكل تهديدًا خطيرًا للزراعة المستدامة والأمن الغذائي. علاوة على ذلك ، أصبحت التغيرات في الظروف المناخية مواتية بشكل متزايد لتربية بعض الكائنات الحية الدقيقة الضارة ، المعادية لزراعة العنب المستدام. أظهرت التغيرات البيئية تأثيرًا سلبيًا متوقعًا على زراعة الكروم من خلال زيادة الضغوط الحيوية وغير الحيوية. يمكن استخدام مجموعة من الاستراتيجيات للتخفيف من مثل هذه السيناريوهات ، ومع ذلك ، فإن تكامل الجذور لمكافحة هذه التحديات هو طبيعة مستدامة. أظهرت جذور العنب دورها في التخفيف من المشاكل التي أثيرت بسبب مجموعة متنوعة من الضغوط البيئية. على سبيل المثال ، يتم استخدام بعض أنواع Vitis كجذر ضد phylloxera والآفات الضارة الأخرى للعنب. وبالمثل ، هناك بعض الجذور التي تم تطويرها والتي تتسامح ضد الملوحة والجفاف والبرد والحديد الكلور. مع الظروف البيئية المتغيرة باستمرار ، ليس من الضروري أن يعمل الجذر الأساسي بشكل أفضل في مكان معين بشكل جيد في مكان آخر. في هذه المقالة ، تم استعراض العديد من جذور العنب التي لها خصائص مقاومة أو تحمل محددة ضد مجموعة متنوعة من الضغوط ، بما في ذلك الآفات والأمراض والملوحة والجفاف. تعتبر المساعي المتناسقة في تحسين جذور العنب والاستفادة منها حاسمة لاستدامة صناعة العنب ، لا سيما خلال الضغوط البيئية المتزايدة باستمرار.

لكلمات المفتاحية: الجفاف ، phylloxera ، الملوحة ، الجذور ، زراعة الكروم ، الإجهاد

Introduction

Grape (Vitis vinifera L.) is one of the top fruit crops of the world that are grown for liquid (juice, vinegar, spirits), dry fruit (raisin) and fresh fruit consumption. At present, climate change is one of the major global concerns affecting viticulture (Sara et al., 2018) and adversely impacting grapevines growth and development, thus affecting its yield and quality (Dinis et al., 2018). The use of appropriate rootstock is a long-term strategy in changing climatic conditions (Fraga et al., 2016; Cramer et al., 2011). Hence, the grapevine rootstocks have their significant importance as they can mitigate environmental stresses by adapting to adverse soil and climate conditions. An ideal rootstock should have abilities to withstand drought, heat, salinity, cold, insect and pest resistance with the ability to adopt a wide range of soils (Ollat et al., 2015). Besides, a rootstock should have abilities of early bearing and high yield attributes, however, it is seldom that single rootstock possesses all desirable traits (Theodore and Stephen, 1997).

Before selecting and propagating a grapevine rootstock, a number of its attributes and characteristics are evaluated. Traits such as its compatibility, vigor, yield, rooting ability, and propagation technique (Pedersen, 2006), adaptation to the soil, and climate are taken into consideration (Pavloušek, 2011). Moreover, these rootstocks should have tolerance against salinity and drought with the ability to resist soil-borne pathogens. In grapes, there is the problem of graft incompatibility, which occurs over the time (Gökbayrak et al., 2007). This

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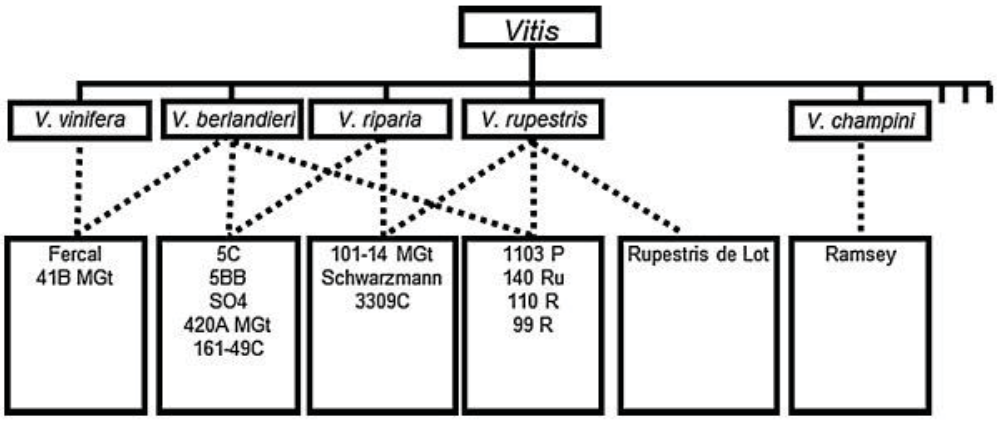

Figure 1. The genetic origin of some rootstocks used worldwide [adapted from Dry (2007)]

incompatibility results in poor vascular bundle development and causes phloem degeneration (Cookson et al., 2013). This problem resulted an uneven distribution of water and nutrients with poor plant performance (Pina et al., 2009).

In vineyards establishment, identification of the most suitable rootstock choice is one of the key factors for its success (Loreti et al., 2006). The rootstocks of grapes play a key role in dividing plant biomass between trunk, root, shoot, and fruit (Köse et al., 2014). The carbohydrates stored in canes are responsible for plant health and vigor of earlier year growth. Similarly, the carbohydrates stored in the plant roots are responsible for root and shoot development. It also increases stem growth, initiates flower bud induction, and fruit setting (Göktürk et al., 2005). Besides, rootstocks are responsible for controlling the scion flowering and fruiting. It also affects the quality of the fruit (Reddy et al., 2003). Moreover, grapes rootstocks have exhibited the ability to withstand biotic stress like phylloxera and abiotic stresses including drought, salinity and flooding (Satisha et al., 2007). At present, climate change is of great concern as erratic environmental conditions are making agriculture difficult to sustain, because of heavy rain, flood or drought, soil and water salinity, and a range of extreme weather conditions. The grapes grown under (light and water) stress conditions resulted in low yield as compared to grapes grown under ideal environments (Akram et al., 2019).

Most of the grape rootstocks are developed from North American Vitis species. Approximately 90% of grape rootstocks that exist in the world were developed from ten rootstock cultivars (Keller, 2010). The genetic origin of grape rootstock used broadly in the world is shown in (Figure 1). In viticulture, it is essential to understand the behavior of rootstocks as the success of viticulture depends upon various factors including soil, climate, biotic-abiotic factors and rootstock -scion combinations. Any change in these factors can entirely change the scenario. There are certain rootstocks in grapes that are capable of overcoming the biotic and abiotic stresses at large. Therefore, it is essential to find such rootstocks in grapes that carry the desirable traits to withstand the ever-increasing environmental stress. Thus, the objectives of this study were to review the current literature on the role of grapes rootstocks in mitigating environmental stresses in sustainable viticulture production.

Response of Grape Rootstocks to Biotic Stresses

Phylloxera

The "phylloxera" species is one of the most destructive pests of grapes. It consists of several species. In the 1880s, its first species (Phylloxera vitifoliae Fitch.) appeared and it was a root parasite (Coombe, 1999). It severely affected the grapes growing areas. Then in 1930, grapes first rootstock was developed to tolerate the attack of "phylloxera", which in the 19th century destroyed several European vineyards (Granett at al., 2001). The use of rootstock in viticulture was initiated due to this pest. To cope with phylloxera, the grapes rootstock from North American Vitis species were selected and European varieties were grafted (Vrsic et al., 2016). Later grape hybrids were used to solve this problem. But the phylloxera species (Daktulosphaira vitifoliae Fitch) became a dynamic and more aggressive strain, which destroyed several vineyards of Europe. American rootstocks showed their resistance against this pest.

After the 1990s, the European rootstocks were replanted again and were grafted on "Borner" rootstock (Becker, 1989). This rootstock has shown strong resistance against phylloxera and since it is used commercially (Blank et al., 2009). This rootstock was a hybrid

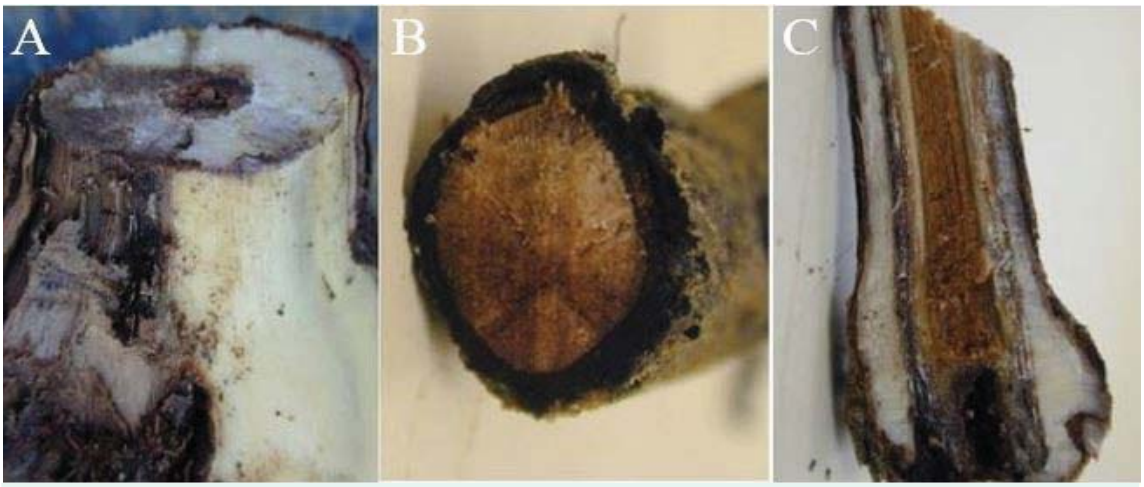

Figure 2. The cross-section of rootstock infected by black foot disease (A), cross-section of the root (B) and longitudinal-section of a young grapevine infected with Cylindrocarpon spp. (C) [adapted with the permission from Halleen et al. (2007)].

that was obtained by the crossing of two grape species "Vitis riparia 183 Gm × Vitis. cinerea Arnold" (Ambrosi et al., 1994). At present, several rootstocks are being developed in Europe, which showe their resistance against phylloxera (Arrigo and Arnold, 2007). The highest resistance against phylloxera has been observed in American species, but its mechanism of resistance is still not clear. The American species that showed resistance against phylloxera includes: Vitis Rupestris, Vitis riparia and Vitis berlandieri. Most of the grapevine rootstocks are developed from these species show a very less genetic variability among them. Serra et al. (2013) reported that about 90% of grape genotypes are grafted on ten rootstocks, which shows that the grapes are at huge risk and these rootstocks can lose their significance with time being due to less genetic diversity. For example, A × R1 Californian rootstock which was the combination of Vitis vinifera ×Vitis rupestris has lost its root system significance and it is further not effective (Corso and Bonghi, 2014). Until now, the most resistant hybrids against phylloxera developed from Vitis Rupestris, Vitis riparia and Vitis berlandieri species are 101-14, 196.17 Castel and Schwarzmann (Grant and Matthews, 1996). Similarly, another grapevine rootstock SO4 (Selection Oppenheim 4) showed its highest resistance against phyloxera (Schmid et. al., 1998). However, there is always a great need to develop more rootstock against this pest.

Black Foot Disease

In 1961, black foot disease was recorded for the first time in France (Maluta and Larignon, 1991). After that, this disease was observed in the vineyards of Tasmania (Sweetingham, 1983), Portugal (Rego et al., 2000) and the USA (Gugino and Travis, 2003). The causal organism isolated for this disease was Cylindrocarpon species, which was a soil-borne pathogen (Francois et al., 2006) and this affects the roots of grapes (Brayford et al., 2004). In the black foot, several species of Cylindrocarpon are involved, namely C. destructans, C. obtusisporum, C. macrodidymum and C. fasciculare. But the most destructive one was the C. destructans (Halleen et al., 2004). Recently this disease is also reported with fungal species of genera "Dactylonectria" and "Neonectria" (Lombard et al., 2013). It is called "black foot" as it causes brown to black streaks or black discoloration at the main base of the rootstock. The damage caused by this disease on grapevine rootstock is presented in Figure 2.

This disease causes contraction in root biomass with deep necrotic lesions on root hairs (Agustí-Brisach and Armengol, 2013). It also affects plants aerial parts by weakening vegetation, bud breaking, uneven wood maturity and interveinal chlorosis (Larignon, 2004). This disease is most common around the coastal area where it is known as "aka young vine decline". It affects the roots of mature and young vines (Bleach et al., 2007). This disease can be controlled by planting the resistant rootstock against this disease or by controlling the disease management practices at the nursery level. In grapes rootstock, the least susceptible rootstock reported against this disease was 101-14 while the most susceptible rootstock to this disease was Riparia Gloire (Brown et al., 2013). The other resistant rootstocks reported against this disease are 'O39-16' and 'Freedom', which were taken from the species Vitis riparia (Gubler et al., 2004). While in the coastal areas, the rootstock A X R1 (Aramon Rupestris Ganzin No. 1) showed the highest resistance against this disease as compared to 140R and 039-16 (Battany, 2015).

Petri Disease

Petri disease is the most destructive disease of newly established vineyards, especially which have less than ten years' lifespan. This disease is reported by several countries around the world (Chicau et al., 2000; Crous

Table 1. Grapevine rootstocks tolerance reported to different biotic stresses (Phylloxera, Black foot disease, Petri disease, Crown gall and Nematodes)

Rootstocks	Phylloxera	Black foot disease	Petri disease	Crown Gall	Nematodes	References
196.17 Castel	High	-	-	-	-	Corso and Bonghi (2014)
Schwarzmann	High	Low	High	-	Medium	Corso and Bonghi (2014); Eskalen et al. (2001)
101-14*	High	-	-	-	-	Brown et al. (2013)
A X R1**	Low	High	High	-	-	Battany(2015); Eskalen et al. (2001)
Riparia Gloire	Medium	High	Medium	High	-	Corso and Bonghi (2014); Ferreira et al. (2018)
Freedom	Medium	High	-	Low	High	Corso and Bonghi (2014)
1103 Paulsen	Medium	Low	Medium	-	Medium m	Corso and Bonghi (2014); Ferreira et al. (2018)
O39-16	High	High	-	-	Low	Gubler et al. (2004)
Ramsey	High	-	-	High	High	Davut et al. (2018)
110-R	-	-	High	High	-	Davut et al. (2018); Eskalen et al. (2001)
SO4***	High	-	Low	Low	Medium	Schmid et al. (1998); Ferreira et al. (2018)
Kober 5 BB	Medium	-	-	High	Medium	Demir et al. (1998)

Full name of rootstocks: 101-14 (101-14 Millardet et de Grasset); A X R1** (Aramon Rupestris Ganzin No. 1); SO4*** (Selection Oppenheim 4)

and Gams, 2000). This disease is also called young vine decline or black goo decline (Gubler et al., 2004). It is a fungal disease and is mostly associated with P. Phaeoacremonium spp. (Gramaje and Armengol, 2011), and with other fungi Cephalosporium and Acremonium (Larignon, 2012). This disease shows both external and internal symptoms. The affected plant has undersized trees, having less vegetation and chlorotic leaves with necrotic borders. While internally, it causes brown necrosis and results in the formation of phenolic compounds, gums and tyloses around the xylem tissues (Gramaje and Armengol, 2011). The sap flux appears during necrosis and it is known as "black goo" (Larignon, 2012). The pathogens associated with this disease are soil-borne pathogens, and it is directly affecting the roots of the plant.

The incidence of this disease varies with rootstocks susceptibility. The rootstock "Freedom", "1103" and "SO4" are highly susceptible to this disease while the rootstocks "Salt Creek", "St George", "Harmony", "110R", "3309C", "Schwarzman" and "A X R1" and proved least susceptible to Petri disease (Eskalen et al., 2001). In another finding, the rootstock "Golia" showed its highest resistance against Petri disease. While the rootstocks "SO4", "Riparia Glorie" and "1103" were moderately tolerant, whereas the rootstock "IAC 572" was highly susceptible to this disease (Ferreira et al., 2018).

Crown gall

Crown gall is one of the most destructive diseases of grapes and it limits the grape production worldwide (Burr et al., 1997). The causal organism of this disease is bacteria "Agrobacterium vitis". This pathogen affects the main trunk and canes of the grapevine. This pathogen induces small galls on the infected part of the vine. The incidence of the disease started with the mechanical or with the frost injury at the sites. After the injury, small galls start to form on the infected parts. With disease severity, the galls start to enlarge. In most destructive cases, the pathogen disturbs the graft union, internal disturbance of the plant systematic system, or even death is seen in grape growing regions.

The disease incidence is normally reduced by good cultural practices along with effective bio-control and using tolerant rootstock. In grapes, Vitis raparia is considered as a tolerant species against this disease (Süle and Burr, 1998). Other rootstocks (i.e. Ramsey, 110-R and 1613-C) are tolerant against this disease (Davut et al., 2018). The incidence and variability of the disease vary with the climate of a region and rootstocks used. For example, "Chardonnay" and "Riesling" showed resistance in Chile, while these two genotypes were found susceptible in different regions of the USA (Burr et al., 1997). Similarly, the rootstock "Ramsay" was found highly resistant to this disease in Turkey, while it was found susceptible in South Africa (Davut et al., 2018). In another finding, the rootstocks "Kober 5 BB" and "Ramsey" were found moderately tolerant to crown gall disease (Demir et al., 1998).

Nematodes

In grapevine, root-knot nematode is another common destructive pest. In America, this pest reduces 20% of the total yields of grapevines and these nematodes are becoming a serious problem of Australian viticulture (Nicol et al., 1999). There are several common nematode species available in vineyards, such as Meloidogyne incognita, M. arenaria, Pratylenchus vulnus and M. javanica (Mckenry and Safdar, 2006). All nematodes species can penetrate deeply into the root of a plant for taking their nutrients. There are two types of nematodes, endoparasitic and ectoparasitic. Some species are endoparasitic nematodes entered into the root of the plant and consume nutrients from the root, while the ectoparasitic nematodes live outside the plant root and consume nutrients from outer tissues of the roots.

In grapes, the most common and destructive root-knot nematodes belong to the genus Meloidogyne. These nematodes are sedentary endoparasites, which hatch eggs at the second stage of juvenility and then migrate from soil towards the root of grapevine roots. After penetration in the roots, they form giant cells where it completes its next juvenile stages. A single gall or giant cell can consist of one to several females which can lay up to 1500 gelatinous matrix eggs (Brown et al., 1993). The best way to control nematodes is the use of resistant rootstock. In Australia, Ramsey rootstock is specifically used to control Meloidogyne species nematodes, while the rootstocks, V. champini, V. longii and V. cinerea have their resistance against root-knot other nematodes species (Nicol et al., 1999). Normally a single rootstock has resistance against single species, but now several developed rootstocks show their resistance against more than one nematode. Recently developed rootstocks RS-3, RS-9, USDA 6-19B, USDA 10-17A and USDA 10-23 Band have their resistance against more than one nematode species (Anwar and McKenry, 2006; Gu and Ramming, 2005). Recently, five grape rootstocks UCD GRN1, UCD GRN2, UCD GRN3, UCD GRN4 and UCD GRN5 are developed, which show their resistance against Paratylenchus hamatus, Mesocriconema xenoplax, Tylenchulus semipenetrans and Pratylenchus vulnus nematodes (Ferris et al., 2012). The specific rootstocks have their specific tolerance against each biotic stress (phylloxera, black foot disease, Petri disease, crown gall and nematodes) and these are shown in Table 1.

Response of Grapes Rootstocks to Abiotic Stresses

Salinity

Salts are present in the form of minerals and these are required for the growth of plants. These salts are readily available in soil and water. Every plant species has its specific potential to tolerate salt. The capacity of a plant to withstand or endure excessive salt in its root zone is called "Salt tolerance" (Adnan, 2004). Deficiency or toxicity of minerals, both are harmful to plants. The excessive salts present in the soil are called "salinity". Saline soils contain excessive amounts of salts (Na^+ and Cl^-) present in the soil that are harmful to grape growth and yield (Corso and Bonghi, 2014). In grapes, high salinity level causes disturbance in water and minerals uptake (Ismail et al., 2012). Grape genotypes are moderately tolerant of saline conditions however prolonged exposure to salt stress especially the chloride ions are highly damaging. These ions disturb the CO_2 assimilation and show effects on the stomatal conductance of grapes by its osmotic pressure (Cramer et al., 2007). Grapes growing in semi-arid irrigated areas are highly affected by this problem. The saline area of the world is increasing day by day and around 40% of world arable land is under saline conditions (Nabati et al., 1994).

Several factors are affecting soil salinity. It includes water or irrigation systems, the climate of a particular area, soil of grapes cultivated area and the genotypes, especially the rootstock–scion combination used for commercial cultivation. It is also reported that grapes rootstocks are more affected by Cl^- ions as compared to Na^+ (Cramer et al., 2007). The grapes rootstocks have great variability in taking up of these ions and their accumulation concentration varies with the selected rootstocks (Fisarakis et al., 2001). The wild species grapes (i.e. Vitis rupestris) have the maximum strength to exclude Cl- ions. Similarly, the grapevine's other rootstocks Vitis cinerea and Vitis champini tolerate saline conditions. For the good production of grapes, the combination of scion and rootstock is very essential. Vitis berlandieri had great strength to withstand salt but when vinifera scion was used. It reduces the strength of Na^+ and Cl^- expulsion. For example, a hybrid 41B which was the combination of Vitis berlandieri × Vitis vinifera lost its ability to exclude ions. Other reported grapes rootstocks (Ramsey and two hybrids, 1103 Paulsen and R2) showed a positive response towards salinity and increased the weight and bunches number (Walker et al., 2002). While Southey and Jooste (1992) recommended 101-14 Mgt and 143-B Mgt rootstocks against salinity. In a comparison of 101.14 and M4 as commercial rootstocks, M4 showed greater capacity to tolerate saline and drought stress by maintaining the physiological processes and photosynthetic activity (Meggio et al., 2014). Scion/rootstock grafting interaction showed that a sensitive variety (i.e. Syrah) grafted onto a moderately tolerant rootstock (i.e. 1103P) resulted in enhanced tolerance levels against salt stress as compared to a moderately tolerant variety (i.e. Muscat d'Italie), which was grafted on a sensitive rootstock (SO4) (Hanana et al., 2015).

Drought Stress

Climate change is one of the major threats and an increase in temperature on the landmass is causing the

problems of drought and water scarcity. Among abiotic factors, drought is one of the main factors that directly affect the yield and productivity of a crop (Tsago et al., 2014). Based on climate models and weather predictions, it is assumed that there is an increase in arid land in the future (Dai, 2013). The solution to this problem is to use such genotypes that are greatly water efficient. This is one of the breeding key strategies for the improvement of genotypes (Marguerit et al., 2012).

In grapes, there are certain rootstocks, which use water more efficiently. Hence, the rootstock can play an effective role in drought by improving water efficiency. The efficacy of rootstock depends upon several factors, such as scion, vigor, stomatal conductance, aquaporin proteins and their combinations. In water stress conditions, stomatal conductance plays an effective role in water regulation and first organelles to respond to drought (Damour et al., 2010). Moreover, during stress conditions, a plant releases an abscisic acid hormone, which accumulates in grape leaves and this retards the plant cellular growth (Serra et al., 2013). This hormone is immediately released by the plant when it is in stress and the accumulation of abscisic acid in leaves causes closure of stomata. It is also observed that in water stress conditions, there is a production of aquaporin genes, which controls the water use efficiency of plants and these genes are more in drought-tolerant rootstocks.

Considering drought conditions, the rootstocks are divided into two categories: (i) the rootstocks having higher vigor and drought tolerance mechanism, (ii) rootstocks having least vigor and drought tolerance. The rootstocks exhibit higher vigor; they develop rapid roots growth in a later season, especially during wet conditions while rootstocks having less tolerance and develop roots in the early growing season without prevailing any wet conditions (Serra, 2013). In grapes rootstocks, it is essential to find both drought tolerance mechanisms to cope with water-scarce conditions. Plants may suffer metabolic changes due to exposure of abiotic stresses and these result in the decline of quality and productivity of grapevine. Rootstock integration approach can be used to mitigate such harmful impacts due to their abilities to enhance the drought tolerance mechanism during the scion. Under deficit water regimes, the grafter grapes rootstocks (i.e. Mgt 101-14 and 1103 Paulsen) showed significant alterations in grape technological maturity. The primary metabolism was not noticed in the rootstocks, while the accumulation of phenolic compounds in berries (e. g. anthocyanins) was very distinct. Plants under water stress and normal water regimes showed a significant difference in the gene and miRNA expressions. Results conferred that the rootstocks can modulate water stress effects on grapes through regulating the secondary metabolism (Zombardo et al. 2020).

Several grapevine rootstocks showed their variability in drought tolerance. In grapes, the highest drought tolerance was shown by V. champinii species (Padgett-Johnson et al., 2003). Early 1935, two rootstocks 'Riparia' and '101-14Mgt' were commonly used against drought (Dry and Coombe, 2005). After another rootstock "Ramsey" of V. champinii became very famous and was widely used in Australian vineyards due to its highest drought tolerance (Walker and Clingeleffer, 2009). The other grapevine rootstocks (i.e. Kober 5BB, 140 Ruggeri, Lider 116-60, 1103 Paulsen and Richter 110) showed their tolerance towards drought (Flexas et al., 2009). M4 rootstock planted at water-deficient and salt stress soil showed its tolerance towards salt stress and water stress. It also maintains its photosynthetic activity. In another finding, the rootstock '110R' showed the highest drought tolerance while '101-14Mgt' showed the medium tolerance, whereas 'Riparia' showed the least tolerance to drought (Ollat et al., 2015). Similar results were reported by Pouget and Delas (1989) and it showed that 'Riparia' and '101-14Mgt' were low in drought tolerance as compared to '110R'.

In other studies, it was reported that the level of aquaporin genes was different in grapes roots and leaves. The concentration of this gene was less in leaves where it reduced the transpiration rate, while its concentration was more in roots and it promotes roots elongation for water uptake (Galmés et al., 2007). In a hot and dry climate, rootstocks having vigor extends greater as compared to less vigorous rootstocks. Similarly, the grapevines grafted on 1103P rootstock showed a deep root system for water uptake during water stress as compared to 101-14 rootstock and it showed less depth during summer (Alsina et al., 2011). In grapes, certain hybrid rootstocks were developed with the combination of grapes xerophylic species V. rupestris. It was observed that the combination of V. berlandieri × V. rupestris was highly drought-tolerant (Tramontini et al., 2013). Similarly, the scions grafted on drought-tolerant rootstocks showed good evaporation, transpiration, carbon assimilation and water conductance (Alsina et al., 2011).

Low Temperature Stress

Low temperature is one of the major environmental constraints affecting grape production. Most cultivars growing in different geographical parts of the world belonging to European origin showed poor cold resistance (Yu et al., 2017). Low temperature disturbs the physiological and biological function of plants. In extreme cases, low temperature causes problems like crown gall or in severe situations, it may kill the whole grapevine. In grape genotypes, there is a minute difference in temperature of 1 to 2°C for cold tolerance, but this small difference is essential for vine survival. Because each genotype has a certain capacity to tolerate freezing temperature, especially in dormant seasons. In low temperatures, grapes cane showed cytoplasmic desiccation, bud freezing, and primordial death, especially in late winter (Anne, 2004).

The grapes rootstocks have their direct effect on freezing tolerance and scion biochemistry, whereas it

Table 2. Grapevine rootstocks tolerance reported to different Abiotic stresses (Salinity, Drought, Iron chlorosis, Low-temperature stress)

Rootstocks	Salinity	Drought	Iron chlorosis	Low temperature	References
196.17 Castel	High	High	-	-	Corso and Bonghi (2014)
Schwarzmann	Medium	Medium	-	-	Corso and Bonghi (2014)
101-14*	Low	Medium	Low	-	Alsina et al. (2011)
Riparia Gloire	Medium	Low	-	Low	Corso and Bonghi (2014); Ollat et al. (2015)
101-14Mgt	High	Medium	Low	-	Ollat et al. (2015); Southey and Jooste (1992)
Freedom	Low	Medium	Low	Medium	Gubler et al. (2004)
110R	-	High	-	-	Ollat et al. (2015)
1103 Paulsen	High	High	-	Medium	Corso and Bonghi (2014)
O39-16	High	-	-	-	Corso and Bonghi (2014)
Ramsey	High	Medium	Medium	-	Flexas et al. (2009)
3309C	-	-	High	High	Hoover et al. (2002)
SO4**	-	-	Medium	Low	Bavaresco and Lovisolo (2000)
Kober 5 BB	Low	Medium	-	High	Flexas et al. (2009)

Full name of rootstocks: 101-14 (101-14 Millardet et de Grasset); SO4** (Selection Oppenheim 4)

has an indirect effect on vine size (Striegler and Howell, 1991). The grapevine rootstocks are made up of genus 'Vitis' having several species. Each species has its specific cold tolerance. In laboratory conditions, V. rotundifolia tolerated -20 to -23°C, whereas in field conditions it can tolerate up to -13°C freeze (Clark and Watson, 1998). In grapes, Couderc 3309 (C3309), Kober 5BB (5BB) and Selection Oppenheimer No. 4 (SO4, V. spp.) are widely used rootstocks of cold regions as they can tolerate the low temperature. However, the performance of scion grafted on these rootstocks varies with the individual rootstock (Anne, 2004). In field conditions, the rootstock "C3309" acclimated more rapidly as compared to others, while rootstock "5BB" was least acclimatized rootstock (Miller et al., 1988). Similarly, another rootstock used in Eastern US is 3309 Couderc (C-3309). It is the combination of V. riparia and V. rupestris. It is a cold-hardy rootstock having tolerance against phylloxera and acidic soils as well (Hoover et al., 2002). The temperate region fruit rootstocks must contain the character of winter or cold hardiness (Nimbolkar et al., 2016).

Iron Chlorosis

The calcareous soils usually have iron (Fe) deficiency, leading to grapevines grown on calcareous soils having iron chlorosis. The grapevines are also sensitive to the calcareous soils, especially when these are rich in bicarbonates compounds. In grapevines, iron chlorosis causes stunted growth of the vine with low yield. Further, it also affects plant longevity and productivity (Covarrubias and Rombolà, 2013). During iron chlorosis, the production of Fe-reductase enzyme increases in grapevines and the plant excretes organic compounds and protons in its roots, thus resulted in increased Fe solubility and lowered pH. This condition of the plant is known as a strategy I (Jiménez et al., 2007).

In response to iron chlorosis (calcareous soils), grape rootstock (140 Ruggeri) is proved more efficient as it has not shown signs of iron deficiency. The high tolerance against iron deficiency was observed in this rootstock due to Fe (III)-reductase activity in its root. Moreover, this rootstock releases toxic phenolic compounds to the soil when planted in calcareous soils (Ksouri et al., 2006). The Fercal rootstock in France and 140 Ru (Vitis berlandieri × Vitis rupestris) rootstock in Italy, showed its highest tolerance against lime chlorosis (Fregoni and Bavaresco, 1986). The scion and rootstock combination is also necessary when planted at calcareous soils. The grape rootstocks 3309C showed a positive response when Pinot blanc cultivar was grafted on it (Bavaresco and Lovisolo, 2000). Similarly, the rootstock SO4 showed its medium tolerance against iron chlorosis (Bavaresco and Lovisolo, 2000).

Nowadays, mostly hybrid rootstocks are used against chlorosis. The hybrids showed the highest tolerance against chlorosis when a combination of Vitis riparia, Vitis cinerea and Vitis berlandieri were used. The hybrid rootstocks developed from the combination of Vitis rupestris and Vitis amurensis showed medium tolerance towards chlorosis. For example, the hybrid (Binova × Börner) showed its medium resistance towards lime chlorosis, whereas the rootstock hybrid developed with the combination of Teleki 5C × Börner and [Binova × (Binova × Teleki 5C) × Börner] showed their highest tolerance towards lime chlorosis (Pavlousek, 2009). The specific rootstocks have their specific tolerance against

each abiotic stress (salinity, drought, iron chlorosis, and low-temperature stress) as shown in Table 2.

Conclusion

Globally, climate change is one of the major threats to sustainable grape production since it has shown to have a profound impact on the proliferation of several biotic and abiotic environmental stresses. Rootstocks have demonstrated the abilities to mitigate such stresses through their peculiar evolving plant traits. Several grape rootstocks are developed from Vitis and each species of Vitis has its specific characteristic and tolerance mechanism against specific ranges of stress. Grape rootstock responses to biotic and abiotic limiting factors are multifaceted which involves several ecological, physiological, molecular and genomic mechanisms. Many of these mechanisms have been discussed earlier where several plant traits related to such mechanisms and genomic areas have previously been recognized at the scion and rootstock levels. However, a better understanding of the specific role of alleles in these areas can benefit from manipulating the plant materials to handle the increased risk of biotic or abiotic stresses. In this article, several grapevine rootstocks with their specific resistance or tolerance features against a variety of stresses, including pests, disease, salinity and drought were reviewed. Prolongation of grapevine rootstock improvement through conventional and molecular breeding, extensive evaluation and crop management research is critical for the sustainability of the grape industry as new biotic and abiotic stress factors continued to emerge.

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RESEARCH PAPER

Optimal Density of Asian Seabass (*Lates calcarifer*) in Combination with the Omani Abalone (*Haliotis mariae*), Brown Mussel (*Perna perna*) and Seaweed (*Ulva fasciata*) in a Land-based Recirculating Integrated Multi-trophic Aquaculture (IMTA) System

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تأثيرالكثافة المثالية للقاروص الآسيوي (*Lates calcarifer*) بالاشتراك مع أذن البحر العماني (*Haliotis mariae*) وبلح البحر البني (*Perna perna*) والأعشاب البحرية (*Ulva fasciata*) في نظام متكامل للاستزراع المائي متعدد التغذية (IMTA) قائم على نظام تدوير أرضي

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ABSTRACT. An experiment was conducted to develop a land-based recirculating integrated multi-trophic aquaculture (IMTA) system using a combination of the Omani abalone (Haliotis mariae) and Asian seabass (Lates calcarifer) as fed species, brown mussel (Perna perna) and seaweed (Ulva fasciata) as extractive species. Specifically, this study was carried out to determine the optimal seabass density (20, 40 and 60 individuals per 500-liter tank) on water quality, growth and survival of the cultured species in the system. Sampling of all species was done every two weeks to check their growth. Water samples were taken every two weeks for analysis of ammonia, nitrite, nitrate, phosphate, and silicate. Measurements of temperature, dissolved oxygen and salinity were done daily. Growth of abalone and mussels were higher in fish densities of 20/tank and 40/tank, respectively, while growth and survival of seabass were not significantly different between densities. Biomass of seaweeds decreased during the experiment period. Temperature, dissolved oxygen and salinity were within optimum levels. Ammonia levels decreased as nitrite increased but in some cases it remained high while nitrates did not increase, indicating that nitrites were not converted to nitrates most likely due to the lack of efficient bio-filtration in the mussel tanks.

KEYWORDS: Abalone; seabass; mussel; Ulva; IMTA

المستخلص: أجريت في هذه الدراسة تجربة لتطوير نظام متكامل للاستزراع المائي متعدد التغذية (IMTA) قائم على نظام تدوير أرضي باستخدام أذن البحر العماني (Haliotis mariae) والقاروص الآسيوي (Lates calcarifer) كأنواع تتغذى مع بلح البحر (Perna perna) والأعشاب البحرية (Ulva fasciata) كأنواع استخراجية، وكان الهدف الرئيسي لهذه التجربة هو تحديد كثافة القاروص المثلى (٢٠ أو ٤٠ أو ٦٠ سمكة لكل خزان ذو سعة ٥٠٠ لتر) على جودة المياه ونمو وبقاء الأنواع المستزرعة في النظام. تم أخذ عينات من جميع الأنواع كل أسبوعين للتحقق من نموها، كما تم أخذ عينات المياه كل أسبوعين لتحليل الأمونيا والنيتريت والنيترات والفوسفات والسيليكات، أما درجة الحرارة والأكسجين المذاب والملوحة فقد تم قياسها يومياً. وقد وجد أن نمو أذن البحر وبلح البحر كان أعلى في كثافة الأسماك من ٢٠/خزان و ٤٠/خزان على التوالي، بينما لم يكن نمو وبقاء القاروص مختلفين بشكل كبير بين الكثافات. وقد لوحظ أن الكتلة الحية من الأعشاب البحرية قد إنخفظت خلال فترة التجربة وكانت درجة الحرارة والأكسجين المذاب والملوحة ضمن المستويات المثلى، وقد لوحظ أيضا إنخفاض مستويات الأمونيا مع زيادة النيتريت، لكنها ظلت مرتفعة في بعض الحالات بينما لم تزد النيترات، الأمر الذي يشير إلى أن النيتريت لم يتم تحويلها إلى نيترات على الأرجح بسبب نقص الترشيح الحيوي الفعال في خزانات بلح البحر.

الكلمات المفتاحية : أذن البحر، القاروص الآسيوي، بلح البحر، أعشاب بحرية، النظام المتكامل للاستزراع المائي متعدد التغذية

Introduction

Aquaculture provides socio-economic benefits such as employment, food provision and income generation, but if not done properly, it can lead to adverse environmental impacts which usually come with the development of commercial aquaculture activities (FAO, 2018). By-product wastes in culturing species fed artificial diets in monoculture system are very high (Troell et al., 2003). Environment-friendly aquaculture techniques and systems, for example recirculating aquaculture systems (RAS), are necessary to ensure sustainable aquaculture development. One of the aquaculture systems that has high potential for environmental protection is the integrated multi-trophic aquaculture or IMTA (Neori et al., 2004; Chopin, 2006). IMTA can be sea-based culture system or land-based recirculating system. It involves the culture of a number of species belonging to different trophic levels, some of them are fed while others are extractive, in which the particulate

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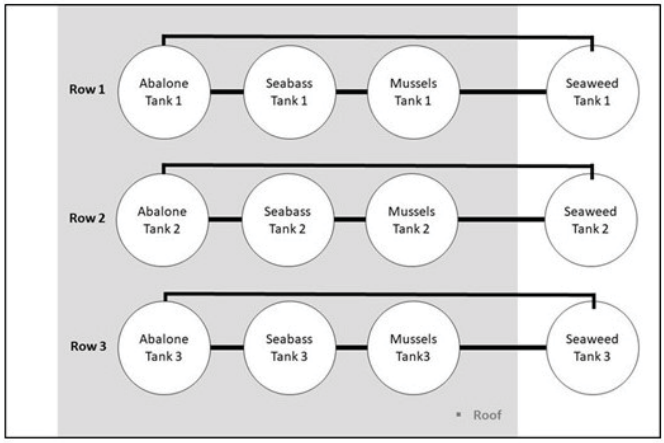

Figure 1. IMTA system of interconnected tanks with recirculating water system. Water from abalone to seaweed tanks flow by gravity while water from seaweed tanks are brought back to abalone tanks by submersible pumps

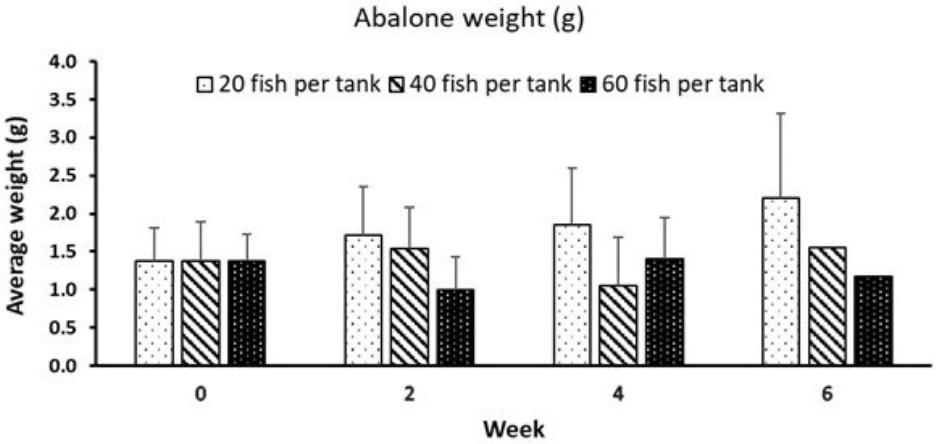

Figure 2. Average weight of abalone in combination with seabass at low (20 fish per tank), medium (40 fish per tank) and high (60 fish per tank) density. Error bars are standard deviation

and dissolved wastes (uneaten feeds, feces, excretion) of other species are utilized by another species.

IMTA requires a careful selection of ecologically and economically important species, some of which can efficiently utilize the wastes from the production of the other species, thus, preventing pollution or eutrophication. In a conventional aquaculture system, wastes go to the environment causing over-enrichment and algal bloom which may eventually cause mass mortalities of cultured and wild species when there is algal die-off and oxygen depletion.

Among the extractive organisms that can be used in an IMTA system are bivalves and seaweeds. Bivalve species such as the mussel Perna perna can filter suspended particles and utilize organic matters in the water (Cheshuk et al., 2003; MacDonald et al., 2011) while seaweeds or macroalgae can take up nitrates which have been converted from ammonia and nitrite by the nitrifying bacteria. Among the seaweeds, Ulva fasciata which is an intertidal green macroalga with high nutrient absorption ability of up to 80% ammonia input (Neori et al., 2000), can be used as the bio-filtration component in an IMTA system (Chopin et al., 2001; Al-Hafedh et al., 2015). In this study, we used the commercially important Omani abalone Haliotis mariae (Al-Rashdi et al., 2008) and Asian seabass or "barramundi" Lates calcarifer as the fed species and the brown mussel Perna perna and seaweed Ulva fasciata as the extractive species.

The Omani abalone is naturally present in the coastal region of the southern part of Oman where it is heavily exploited due to its commercial value. To prevent its depletion, the Ministry of Agriculture and Fisheries Wealth of the Sultanate of Oman is producing juveniles in the hatchery for stock enhancement purposes; however, culturing them in a land-based IMTA system has not been tested yet. If it can be proven that the Omani

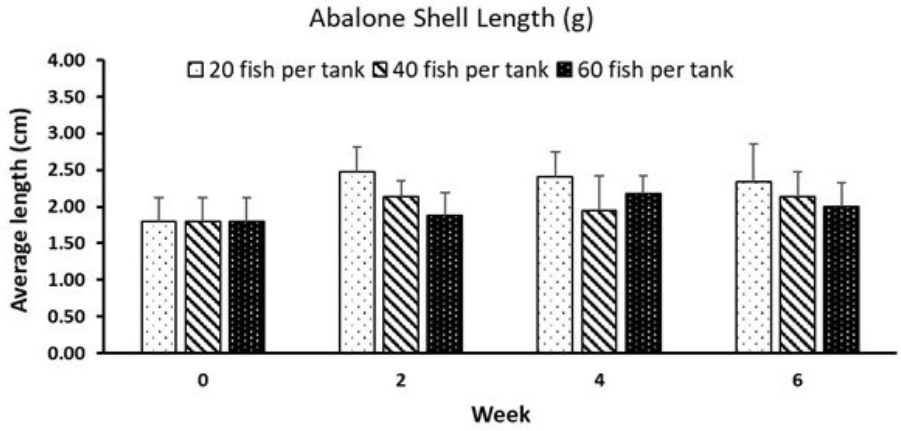

Figure 3. Average shell length of abalone in combination with seabass at low (20 fish per tank), medium (40 fish per tank) and high (60 fish per tank) seabass density. Error bars are standard deviation

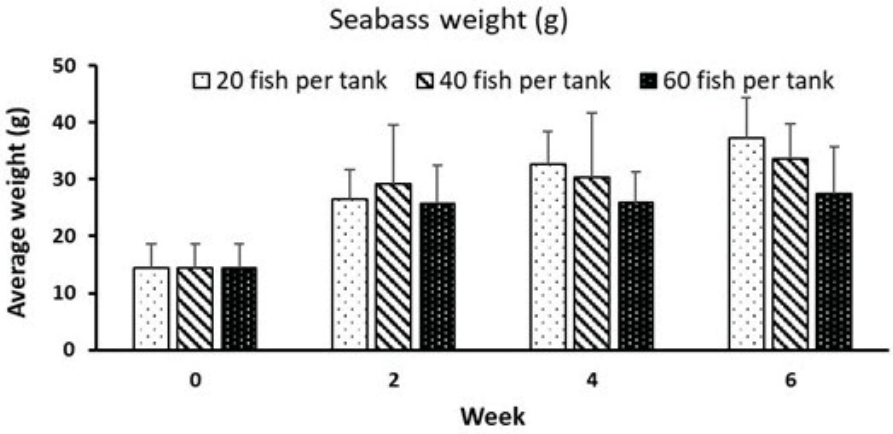

Figure 4. Average weight of seabass at low (20 fish per tank), medium (40 fish per tank) and high (60 fish per tank) density. Error bars are standard deviation

abalone can survive and grow in a land-based recirculating IMTA system, then there is another option for their population to be maintained.

One of the critical factors in a culture system is the stocking density of the cultured species. The objective of the study was to determine the optimal density of the Asian seabass Lates calcarifer as one of the fed species in the land-based recirculating IMTA system, in relation to water quality, and the growth and survival of the cultured species.

Materials and Methods

System Design and Operation

The IMTA system (Figure 1) consists of interconnected 500-l tanks with recirculating water system. Tanks were organized in three rows, each row containing four tanks for the culture abalone (Haliotis mariae), seabass (Lates calcarifer), mussel (Perna perna), and seaweed (Ulva fasciata). Due to lack of tanks and space, replication was not possible.

Initially, 30 individuals of abalone, fish and mussel were selected randomly for weight and length measurements before distributing them to the tanks. The fishes were stocked at 20, 40 and 60 pieces per tank, hereafter designated as low, medium and high fish density. Seaweeds were distributed at 2 kg per tank. The abalone and mussels were distributed at 75 pieces per tank. The experiment was conducted for 6 weeks (42 days).

Abalone, sea bream and mussel tanks were placed under a roof to prevent direct exposure to sunlight and high temperatures, and to minimize water evaporation. The seaweed tanks were placed outside to allow some sunlight needed for photosynthesis but they were covered with a green mesh to minimize direct sunlight and

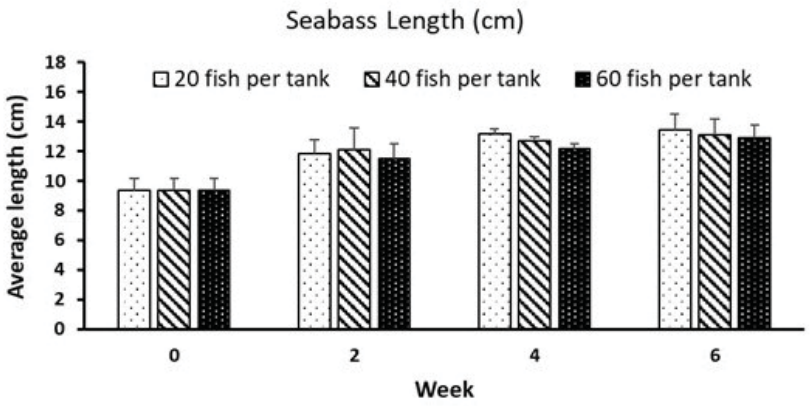

Figure 5. Average length of seabass at low (20 fish per tank), medium (40 fish per tank) and high (60 fish per tank) density. Error bars are standard deviation

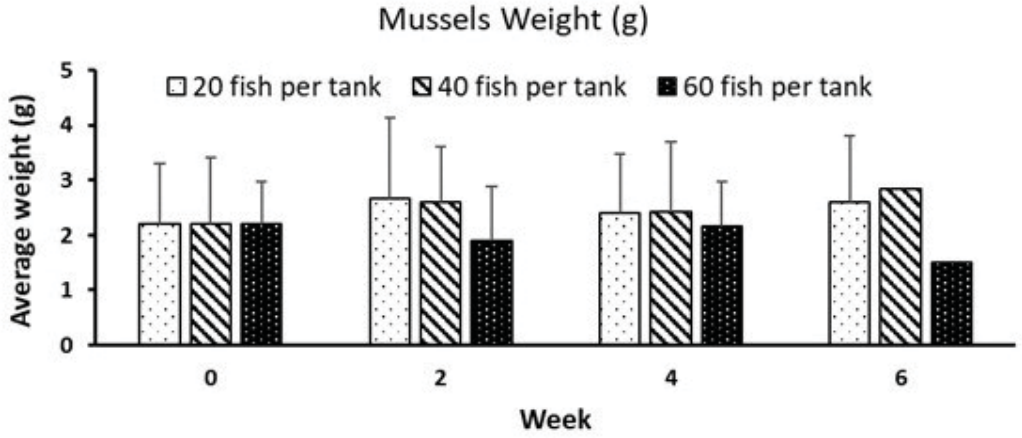

Figure 6. Average weight of mussels in combination with low (20 fish per tank), medium (40 fish per tank) and high (60 fish per tank) seabass density. Error bars are standard deviation

excessive heat. Each seaweed tank was installed with a submersible pump that recirculates the water back to the abalone tank and the rest of the tanks at a rate of 1,200 liters per hour.

The initial feeding rate for seabass was 5% body weight and the amount of feed given to each fish tank was determined by the respective fish density. On the second, third and fourth week, the feeding rate was changed to 3% of body weight and the feed amount was adjusted according to fish density. The abalone were fed with Ulva from the seaweed tanks, approximately 10% of the abalone biomass.

Sampling

Sampling of all species was done every two weeks to check their growth. Water samples were taken every two weeks for measurement of water quality (ammonia, nitrite, nitrate, phosphate, and silicate). Measurements of temperature, dissolved oxygen and salinity were done daily at 8:30 AM and 4:30 PM.

Statistical Analysis

Repeated measures ANOVA was performed to determine any significant difference between treatments, followed by Tukey's test to identify which treatments were significantly different.

Results and Discussion

Growth of Abalone

The average initial weight of abalone was 1.4 g (Figure 2). At low fish density, abalone weight was increased until at the end of experiment while at medium fish density, abalone weight increased on week 2 but decreased on week 4 and increased again on week 6. At high fish den-

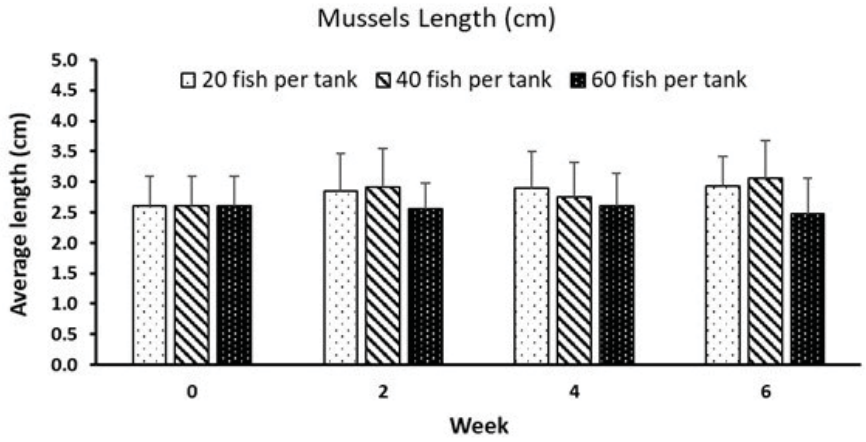

Figure 7. Average length of mussels in combination with low (20 fish per tank), medium (40 fish per tank) and high (60 fish per tank) seabass density. Error bars are standard deviation.

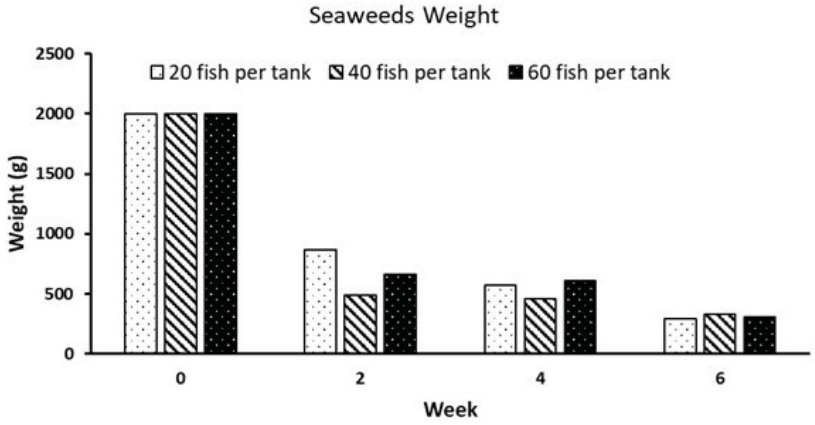

Figure 8. Weight of seaweeds at low (20 fish per tank), medium (40 fish per tank) and high (60 fish per tank) seabass density during the 6-week experiment period

sity, abalone weight decreased on week 2, increased on week 4 and decreased again on week 6. The average final weight was 2.2 g, 1.6 g and 1.2 g, at low, medium and high fish densities of 20, 40 and 60 individuals per tank, respectively. The P-value corresponding to the F-statistic of one-way ANOVA was less than 0.05 which indicated significant difference between treatments. Tukey's HSD test results showed no significant difference in abalone growth between Treatment 1 (low fish density) and Treatment 2 (medium fish density), and between Treatment 2 (medium fish density) and Treatment 3 (high fish density); however, there was significant difference between Treatment 1 (low fish density) and Treatment 3 (high fish density) as growth of abalone in Treatment 1 (low fish density) was higher. At high fish density, water quality was not as good (i.e. high ammonia and nitrite) as in low fish density, therefore, growth of abalone was better in the treatment with low fish density. As shown in the water quality data in section 3.5, ammonia, for example, was lower at low fish density.

The initial average length of abalone was 1.8 cm (Figure 3). At all three fish densities, abalone shell length increased although there were minor fluctuations among weeks. The average final length of abalone in relation to low, medium and high fish density was 2.2 cm, 2.1 cm, and 2.0 cm, respectively, without significant difference among them. Although there was significant difference in abalone weight between Treatment 1 (low fish density) and Treatment 3 (high fish density), there was no significant difference in terms of shell length indicating that body weight and shell length are not proportional or correlated and that weight is a better indicator of abalone growth.

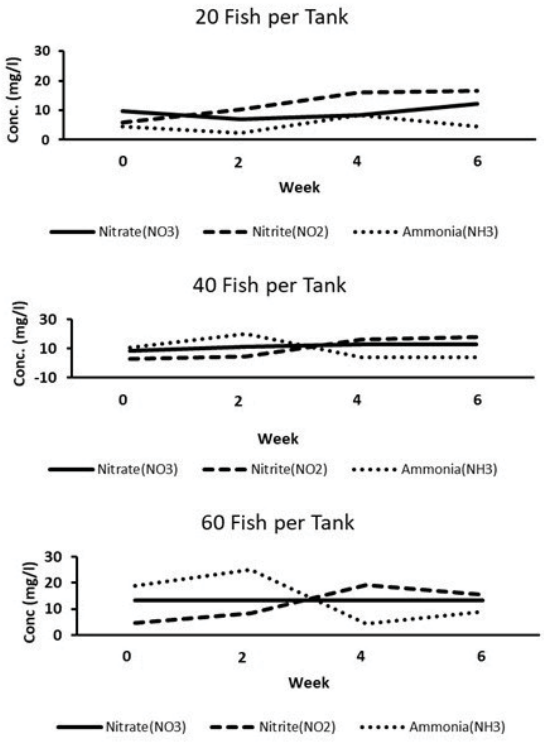

Figure 9. Concentration (mg/l) of ammonia, nitrite and nitrate at low, medium and high fish densities.

Growth and Survival of Seabass

The initial average weight of seabass was 14.4 g (Figure 4). Seabass weight increased from week 0 to week 6. The average final weights at low, medium and high density were 37.2 g, 33.6 g and 27.4 g, respectively. The P-value corresponding to the F-statistic of one-way ANOVA was higher than 0.05, suggesting that the treatments were not significantly different at different fish densities for that level of significance. The Tukey HSD test was also applied and showed the same result.

For commercial culture of sea bass in cages, stocking density of 15-20 fish/m^3 is recommended (Gaitan and Toledo, 2009). In the present experiment, the fish densities (20, 40 and 60/tank) in the 500-liter tanks are equivalent to 40, 80 and 120 fish/m^3 which are higher than the recommended stocking densities. In terms of biomass per cubic meter the initial densities tested are equivalent to 0.58, 1.15 and 1.73 kg/m^3. Ardiansyah and Fotedar (2016) reported that a stocking density of lower than 18.75 kg/m^3 is recommended for culturing in integrated recirculating aquaculture systems.

The initial average length of seabass was 9.4 cm. Its increase during the 6-week culture period is shown in Figure 5. The average final length of sea bass in Treatment 1 (20 fish/tank), Treatment 2 (40 fish/tank) and Treatment 3 (60 fish/tank) were 13.4 cm, 13.1 cm and 12.9 cm, respectively, and were not significantly different ($P>0.05$).

The fish survival rates were 100, 100 and 98% at low, medium and high fish density, respectively. The slightly higher mortality in the high density tank may be due to the relatively higher ammonia concentration observed in this tank. However, since the difference was not significant, this suggests that the fish densities used in the experiment can be also be used in commercial culture even if it is higher than the recommended density for commercial culture of seabass in non-IMTA system, at least for these relatively small fish.

Growth of Mussels

The initial average weight of mussels was 2.2 g. Its growth during the 6-week culture period is shown in Figure 6 with a decrease in mussel weight at high seabass density while at low and medium seabass density, mussel weight increased. The average final weights of mussels in combination with low (20 fish per tank), medium (40 fish per tank) and high (60 fish per tank) fish densities were 2.6 g, 2.8 g and 1.5 g, respectively. The average final weight of mussels in combination with low and medium fish densities were significantly higher than with high seabass densities ($P = 0.026$), indicating that the high fish density did not result in good growth of the mussels. The number of mussels may have not been enough to filter the suspended particles coming from the tanks with high fish density.

The initial average length of mussels was 2.6 cm and its increase in length during the 6-week culture period is shown in Figure 7. The average final lengths of mussels in combination with low (20 fish per tank), medium (40 fish per tank) and high (60 fish per tank) fish densities were 2.9 cm, 3.1 cm and 2.5 cm, respectively. Similar to the data on mussel weight, the growth in length of mussels was not high when combined with fish at high density. This suggests a need to increase the number of mussels in the next experiment.

Growth of Seaweeds

The initial average weight of seaweeds was 2,000 g. Figure 8 shows a significant decrease in biomass at week 2 and onwards. The final weights of seaweeds were 292.5 g, 327.3 g and 304.7 g, respectively, in combination with low (20 fish per tank), medium (40 fish per tank) and high (60 fish per tank) seabass densities.

Initially some seaweeds were taken and fed to the abalone but when their growth was not good, artificial feeds were instead given to the abalone. Overall there was a decrease in the final weight of seaweeds. This could be due to high temperature and the difference of environmental condition in the experiment area (Al-Hail)

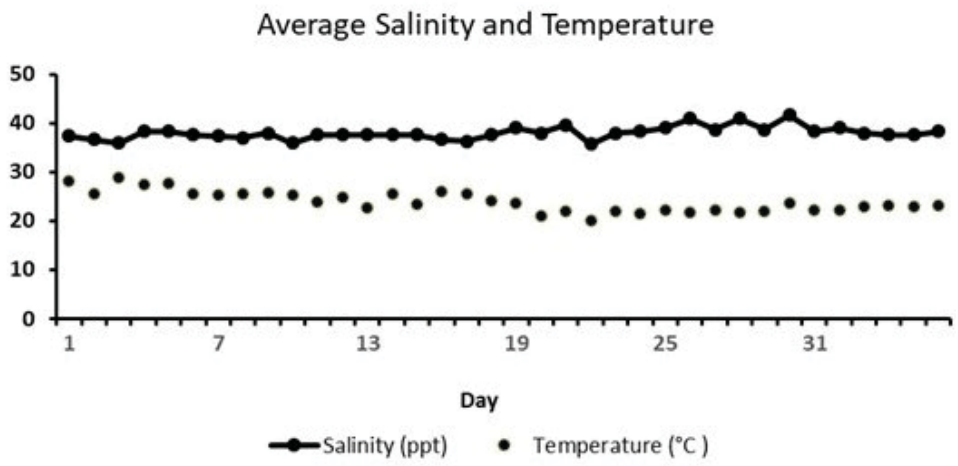

Figure 10. Average temperature and salinity in all tanks during the experiment period.

compared to the origin of seaweeds which were brought from Dhofar region which is usually cooler at temperatures ranging from 21 to 26 °C although the algae were acclimatized for one week prior to the experiment.

The low density of fish resulted in low waste production, thus, low production of nitrates needed for the seaweeds to grow. Yousef et al. (2014) suggested that increasing fish effluent flow in the seaweed culture tanks allows to duplicate the biomass yield. Also, they stated that the increase in water flow is adequate to maintain a high yield and that the stocking rate of 3 kg m^{-3} for Ulva seems to be the best one.

Water Quality

Concentrations of ammonia, nitrite and nitrate in the recirculating system are shown in Figure 9. At low fish density (20 per tank) the concentration of ammonia increased on week 4 and decreased on the week 6, while the nitrite increased on the week 4 and then levelled off and the nitrate increased on week 6. At medium fish density (40 per tank), ammonia increased on week 2 and decreased on weeks 4 and 6 while nitrite increased on week 4 and then levelled off and nitrate was gradually increasing. At high fish density (60 per tank), ammonia increased on week 2 and decreased on week 4 and increased on week 6 while nitrite increased on week 4 and decreased on the week 6 and nitrate was slightly increasing. These three cases indicate the conversion of ammonia to nitrite and then to nitrate but at medium and high fish densities, ammonia build up was earlier (week 2) than at low fish density (week 4).

Temperature and Salinity

Temperature in the culture tanks ranged from 20 to 29°C (Figure 10). At the beginning it was high during summer and then it decreased due to the start of winter season. In seabass culture, optimum temperature for growth and food conversion ranged between 26-32°C (Kungvankij et al., 1984). In our set up, salinity in abalone, seabass, mussels, and seaweed tanks ranged from 35.7 to 41.7 ppt. The reason why the salinity levels fluctuated could be due to addition of fresh water to lower the high salinity levels occurring in some tanks.

Dissolved Oxygen

Dissolved oxygen in seabass tanks ranged from 4.6 to 7.2 mg/l (Figure 11). At the beginning, the dissolved oxygen was high in all the tanks due to clean water used at the start of the experiment. Later on it started to decrease due to the increased production of waste which was acted upon by decomposing bacteria that consumed the oxygen along with the other species. This could also be due to the decrease in seaweeds biomass towards the later part of the culture period while dissolved oxygen level became constant as the waste utilization stabilized.

In the next experiments, we are considering to do the following: (i) increase the number of mussels in the biofilter tank in order to increase the filtration of suspended particles, (ii) add biofilter mat to increase the substrates (in addition to the mussels as substrates) for nitrifying bacteria, (iii) add sea cucumbers in the biofilter tank, for the utilization of detritus and pseudofeces of mussels, (iv) test other extractive species such as seaweeds particularly Gracilaria which have been found to be functioning as a natural filter for ammonia and nitrate (Largo et al., 2016), and (v) test the effect of partial recirculation (8-12 hours only) instead of 24 h recirculation, on water quality, growth and survival of cultured organisms and on the cost and benefit.

Conclusion

There was no significant difference in seabass growth and survival at densities of 20, 40, and 60 per 500-liter tank. However, the highest growth of abalone and mus-

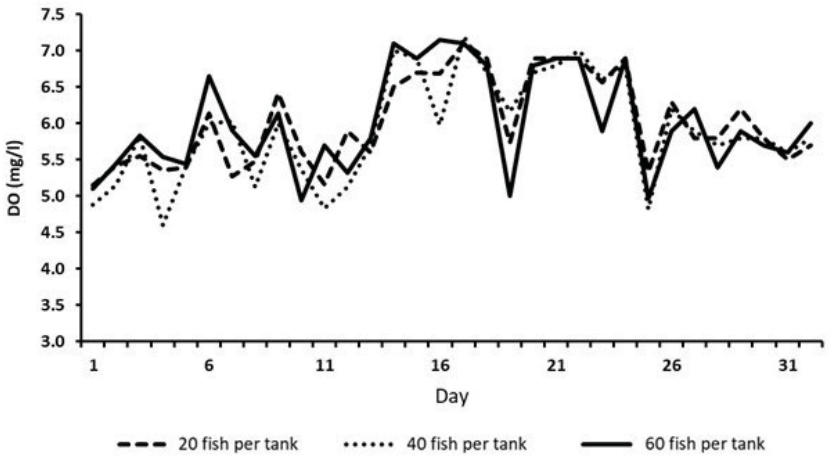

Figure 11. Dissolved oxygen (mg/l) at low, medium and high fish densities.

sels were in low and medium fish density (20 and 40 seabass per tank, respectively). Seaweeds showed a decrease in biomass during the experiment. Water quality parameters, such as temperature and dissolved oxygen were within optimum levels. Ammonia levels decreased as nitrite increased but in some cases it remained high while nitrates did not increase, indicating that nitrites were not converted to nitrates most likely due to the lack of efficient bio-filtration in the mussel tanks. This is the first report on the growth of the Omani abalone Haliotis mariae together with the Asian seabass Lates calcarifer, brown mussel Perna perna, and seaweed Ulva fasciata in a land-based recirculating integrated multi-trophic aquaculture (IMTA) system. Although results may not be highly conclusive due to lack of space and tanks for replication of treatments, the results are useful for further work to validate the findings that will lead to the development of land-based IMTA system especially for the Omani abalone.

Acknowledgements

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RESEARCH PAPER

In vitro Antagonistic Potential, Plant Growth-promoting Activity and Indole-3-acetic Acid Producing Trait of Bacterial Isolates from Button Mushroom (*Agaricus bisporus*) Spent Substrate

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التأثير المضاد، والنشاط المعزز لنمو النبات وميزة إنتاج الأندول ٣- حمض الأسيتيك في المختبر من العزلات البكتيرية من السماد المستهلك (كمبوست) بعد إنتاج فطر المشروم الدائري (*Agaricus bisporus*)

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ABSTRACT. Spent mushroom substrate (SMS) is widely used as a fertilizer and to control plant diseases. The microorganisms surviving in SMS play a crucial role in plant growth promotion and biocontrol activity. In this study, an effort was made to isolate and characterize the bacterial species present in the SMS of Agaricus bisporus and to study their antagonistic potential, plant growth-promoting ability and indole-3-acetic acid (IAA) producing trait. Six different bacterial isolates exhibiting morphological variabilities were obtained from the SMS by serial dilution technique. On the basis of 16S rRNA gene sequences, these isolates were identified as Staphylococcus epidermidis (Sh1 and Sh3), S. aureus (Sh2), Bacillus albus (Sh4), Delftia lacustris (Sh6) and Comamonas aquatica (Sh7). These bacterial strains were assayed for their antagonism against Pythium aphanidermatum, a phytopathogenic oomycete. The results of in vitro dual culture assay revealed that all the 6 bacterial isolates showed low levels of suppression of P. aphanidermatum and recorded less than 5 mm inhibition zone. Among the bacterial isolates, S. epidermidis Sh3 recorded the maximum inhibition zone of 4.2 ± 0.5 mm. Plant growth promotion test using roll paper towel method revealed that C. aquatica Sh7, B. albus Sh4, D. lacustris Sh6 and S. epidermidis Sh3 caused a significant increase in seedling vigour of cucumber compared to control. The seeds treated with the bacterial isolate C. aquatica Sh7 showed the maximum seedling vigor (2018 ± 255). Assessment of in vitro production of IAA by the bacterial isolates revealed that the bacterial isolates highly varied (ranging from 0.28 to 9.25 mg L^{-1}) in their potential for production of IAA. The maximum amount of IAA was produced by C. aquatica Sh7 (9.25 ± 0.02 mg L^{-1}). Further studies are required to assess the possibility of using the IAA-producing bacterial isolates identified in this study or their metabolites to promote plant growth or to enhance growth and yield of mushrooms.

KEYWORDS: Button mushroom; spent compost; IAA production; Agaricus bisporus; antagonistic activity; plant growth promotion.

المستخلص:خلاصة: يستخدم السماد المستهلك (كمبوست) بعد إنتاج فطر المشروم بشكل واسع كسماد وأيضا في مكافحة الفطريات الممرضة للنباتات. تلعب الكائنات الحية الدقيقة التي تعيش في هذا السماد دورًا حاسمًا في تعزيز نمو النبات ونشاط المكافحة البيولوجي. في هذه الدراسة قمنا بعزل وتوصيف البكتيريا الموجودة في هذا الكمبوست الذي يستخدم في إنتاج فطر *Agaricus bisporus* ولدراسة أيضا قدرتها على تثبيط نمو بعض الفطريات وتنشيط نمو النباتات وإنتاج الإندول ٣- حمض الأسيتيك. ست عزلات من البكتيريا ذات صفات ظاهرية مختلفة تم الحصول عليها باستخدام تقنية التخفيف التسلسلي في محتوى تركيز الكمبوست. تم تصنيف البكتيريا بإستخدام التصنيف الجيني في تسلسل وحدة الريبوسومات الموجودة على حمض الريبونيوكليك (S١٦) على إنها (Sh١ و ٣) *Staphylococcus epidermidis* و *S.aureus (Sh2)* و *Bacillus albus (S٤h)* و*Delftia lacustris (Sh٦)* و *Comamonas aquatica (shv)*. هذه السلالات البكتيرية تم تجربتها على تثبيط نمو الفطر الكاذب الممرض*Pythium aphanidermatum* . أظهرت النتائج التي أجريت في المختبر بعد وضع كل من أوميسيتس *P. aphanidermatum* مع جميع العزلات البكتيرية الستة مستويات تثبيط منخفضة وسجلت منطقة تثبيط أقل من ٥ مم. من بين العزلات البكتيرية، سجلت *S. epidermidis Sh*٣ أقصى منطقة تثبيط ٤,٢ ± ٠,٥ مم. كشف إختبار نمو النبات باستخدام طريقة لفافات المناديل الورقية أن *C. aquatica sh*v و *B. albus Sh*٤ و *D. lacustris Sh*٦ و *epidermidis Sh* ٣. تسببو في زيادة معنوية كبيرة في قوة إنبات بادرات الخيار مقارنة بالشاهد. أظهرت البذور المعالجة بالعزلة البكتيرية *C. aquatica sh*v قوة قصوى للشتلات (٢٠١٨ ± ٢٥٥). كشف تقييم إنتاج الإندول ٣- حمض الأسيتيك IAA في المختبر من قبل العزلات البكتيرية أن العزلات البكتيرية شديدة التنوع (تتراوح من ٠,٢٨ إلى ٩,٢٥ ملجرام / لتر) في قدرتها على إنتاج IAA. تم إنتاج أكبر قدر من IAA بواسطة *C. aquatica sh*v (٩,٢٥ ± ٠,٠٢ ملجرام /لتر). هناك حاجة إلى مزيد من الدراسات لتقييم إمكانية إستخدام العزلات البكتيرية المنتجة لـ IAA المحددة في هذه الدراسة أو نواتجهم لتعزيز نمو النبات أو لتعزيز نمو وإنتاج الفطر.

الكلمات المفتاحية: الفطر الدائري، السماد المستهلك، إنتاج Agaricus bisporus، IAA، نشاط تثبيط، تعزيز نمو النبات

Introduction

Mushroom farming has gained recognition in the recent years and has emerged as a promising agro-based business. Malaysia, China, India and Ireland are the world's leading edible mushrooms producers (Hanafi et al., 2018). Several edible mushrooms including button mushroom (Agaricus bisporus), shiitake mushroom (Lentinula edodes), paddy straw mushroom (Volvariella volvacea), oyster mushroom (Pleurotus spp.) and enoki mushroom (Flammulina ostreatus) are being cultivated commercially worldwide (Feeney et al., 2014). Agaricus bisporus is cultivated commercially in Oman. Mixtures of agricultural/poultry/industrial wastes are commonly used as substrates for mushroom cultivation. The mushroom industry discharges huge quantities of spent mushroom substrate (SMS) after harvest. The SMS usually contains mycelia and remnants of fruiting bodies of mushrooms, and the substrate used for cultivation of mushrooms (Kang et al., 2017). A wide variety of biologically active compounds such as extracellular enzymes, antimicrobial compounds and secondary metabolites that are mainly produced by mushrooms are present in the SMS (Kwak et al., 2015). The potential of SMS in large-scale enzymes production, plant diseases control, bioremediation, fertilizer, vermicomposting and for feeding animals has been documented (Inagaki and Yamaguchi, 2009; Ahlawat et al., 2011; Parada et al., 2011; Parada et al., 2012; Kwak et al., 2015; Roy et al., 2015). Several reports indicated the effectiveness of SMS in plant disease management (Yohalem et al., 1996; Uzun, 2004; Goonani et al., 2011; Riahi et al., 2012). Riahi et al. (2012) demonstrated that the extract of SMS inhibited the growth of Lecanicillium fungicola, the causal fungus of dry bubble disease of A. bisporus. Kang et al. (2017) reported that aqueous extract prepared from SMS of Lentinula edodes suppressed the growth of Phytophthora capsici, reduced the Phytophthora blight and enhanced the growth of pepper. The antagonistic microorganisms present in the SMS were attributed to the disease suppression (Riahi et al., 2012). The objectives of the present study were to isolate and characterize the bacterial species present in the spent mushroom substrate of A. bisporus in Oman and to study their in vitro antagonistic potential, plant growth-promoting trait and IAA producing ability.

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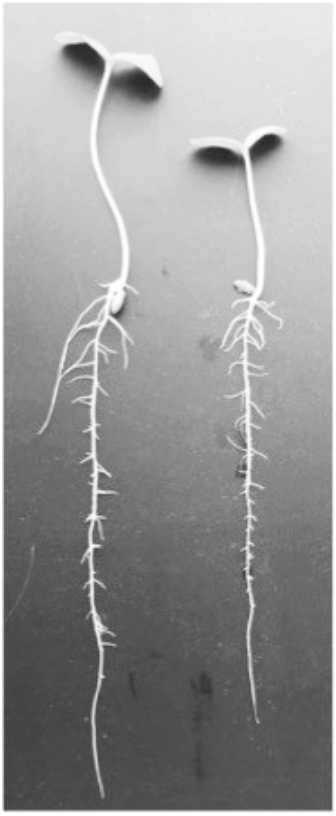

Figure 1. Enhancement of cucumber growth by seed bacterization with Comamonas aquatica Sh7 isolated from spent mushroom substrate of Agaricus bisporus

Materials and Methods

SMS Collection and Bacterial Isolation

Spent mushroom substrate of A. bisporus was obtained from the Department of Plant Sciences, CAMS, Sultan Qaboos University. Bacteria from the SMS were isolated by employing serial dilution plate technique. Briefly, 1 g of SMS was suspended in 99 ml of sterile water and kept on a rotary shaker (150 rpm) for 30 min. Later, the suspension was serially diluted at 1:10 ratio with sterile water. An aliquot (100 µl) from 10^{-4} to 10^{-7} dilutions was gently spread over the Nutrient agar (NA) (Oxoid, UK) with a sterile spreader and then the Petri plates were incubated at 30 °C for 48 h. The bacterial colonies with varying morphological features were selected and transferred to fresh NA plates.

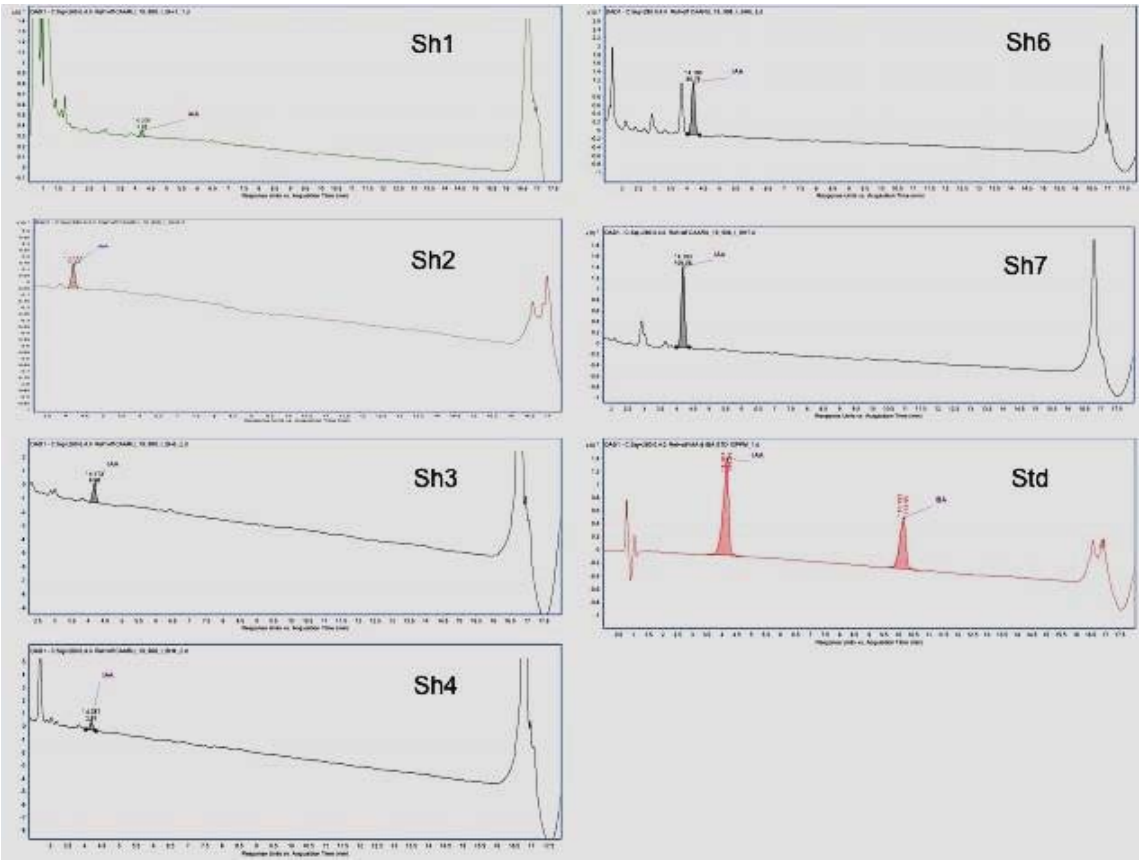

Figure 2. HPLC chromatograms showing IAA produced by bacterial strains from spent mushroom substrate of Agaricus bisporus

Test pathogen

A virulent isolate of Pythium aphanidermatum, the cucumber damping-off pathogen (Al-Shibli et al., 2019), was used in this study. The oomycete pathogen was multiplied on potato dextrose agar (PDA) (Oxoid, UK) at 25 ± 2 °C.

Bacterial Isolates Screening Against *P. aphanidermatum*

The bacterial isolates were screened for their inhibitory effect on P. aphanidermatum using an in vitro dual culture method as described by Al-Hussini et al. (2019). Briefly, a mycelial plug (7 mm diameter) of P. aphanidermatum was placed aseptically on one end of the Petri plate (9 cm diameter) containing PDA. The bacterial isolate was streaked on the other side of the Petri plate (~ 1 cm away from the margin). The inoculated plate was incubated at 27 °C for 3-5 days. After incubation, the inhibition zone was measured. Petri plates inoculated with P. aphanidermatum discs alone were used as control. Four replications were maintained for each bacterial isolate.

Molecular Identification of Bacterial Isolates

The 16S rRNA gene sequence analysis was employed for identification of the bacterial isolates. The bacterial isolates were grown individually on a shaker in nutrient broth (NB) medium (100 ml) at 30°C for 48 h. The bacterial cultures were centrifuged at 14000 g for 15 min and the bacterial cell pellets were collected. DNA was extracted from the bacterial pellet using a commercial foodproof StarPrep Two DNA extraction kit (BIOTECON Diagnostics, Germany). The universal primers 27F (5′-AGAGTTTGATCMTGGCTCAG-3′) and 1429R (5′-TACGGYTACCTTACGACTT-3′) were used for amplification of bacterial 16S rRNA gene by PCR as described by Al-Hussini et al. (2019). The PCR amplified products were sequenced at Macrogen Inc., Seoul, Korea. A database search of homologous sequences was carried out using National Center for Biotechnology Information (NCBI) BLASTN program (http://www.ncbi.nlm.nih.gov).

Table 1. Identification of bacterial isolates from spent mushroom substrate of Agaricus bisporus by 16S rDNA sequence analysis

Bacterial isolate	GenBank accession number	Hit in the NCBI database	% identity
Sh1	MT002750	Staphylococcus epidermidis (KX348319.1)	99.87
Sh2	MT002751	Staphylococcus aureus (CP045468.1)	100
Sh3	MT002756	Staphylococcus epidermidis (LC499612.1)	100
Sh4	MT002776	Bacillus albus (MN793202.1)	100
Sh6	MT002777	Delftia lacustris (MF457528.1)	100
Sh7	MT002779	Comamonas aquatica (MN216294.1)	100

Plant Growth Promoting Activity of Bacterial Isolates

Each bacterial isolate was cultured in NB medium (100 ml) in 250 ml conical flask on a shaker (200 rpm/min) at 30°C for 48 h, and then the bacterial suspension was centrifuged at 3000 rpm for 10 min. The bacterial cell pellet was collected and re-suspended in sterile distilled water and the concentration of the bacterial cells was adjusted to 4×10^8 CFU ml^{-1}. Cucumber seeds (cv. Jabbar, F1; US Agriseeds, USA) were immersed in the bacterial suspension for 3 h at room temperature (25±2 °C), while the control seeds were soaked in sterile distilled water. The roll paper towel method (Shifa et al., 2015) was used to test the effect of bacterial strains on the growth of cucumber. The percentage of cucumber seed germination, seedling shoot length and root length were recorded 12 days after treatment and vigor index was calculated by multiplying the germination percentage of seeds with the total of seedling root length and shoot length. Four replicates of 10 seeds each were used for each treatment.

Analysis of IAA Production

The bacterial isolates were cultivated in NB medium supplemented with 5 mM Tryptophan in a shaker (200 rpm) for 72 h at 30°C. The cultures were centrifuged at 14000 g for 10 min at 4°C and the culture supernatants were collected. The IAA content in the cell-free bacterial culture supernatants was analyzed by High-performance liquid chromatography (HPLC) (Szkop and Bielawski, 2013). Analysis of IAA was performed using a HPLC system (Agilent-1200 Infinity Series), equipped with a high performance autosampler (G4226A), quaternary pump (G4204A), thermostatted column compartment (G1316C) and a diode array detector (DAD) (G4212A). The separation was achieved with Waters Symmetry C8 (5 µm, 3.0×150 mm) column. The mobile phases consisted of A (2.5% acetic acid with a pH 3.8) and B (80% acetonitrile). The mobile phase began with eluent A: eluent B at 80:20 and changed to 50:50, 0:100, 80:20 in 15, 16, and 16.5 min, respectively, and maintained in 80:20 for 1.5 min with a flow rate 1 ml per min. The detection wavelength was set at 280 nm. Peaks in the sample were identified and quantified by comparing with the standard RT.

Statistical Analysis

The experimental design used was completely randomized design. The data on mycelial growth inhibition, percent seed germination and seedling growth of cucumber and IAA production by bacterial isolates,were analyzed by one-way ANOVA (Minitab 17, State College, PA, USA). The data on % seed germination was analyzed after arcsine transformation of values to ensure homogeneity of variance.

Results

Isolation and Characterization of Bacteria from SMS

A total of 6 morphologically different bacterial isolates were obtained from the SMS of A. bisporus. On the basis of 16S rRNA gene sequences, these bacterial isolates were identified as Staphylococcus epidermidis (Sh1), S. aureus (Sh2), S. epidermidis (Sh3), Bacillus albus (Sh4), Delftia lacustris (Sh6) and Comamonas aquatica (Sh7) (Table 1). The 16S rRNA gene sequences of these bacterial isolates were deposited in the GenBank database with the accession numbers MT002750, MT002751, MT002756, MT002776, MT002777 and MT002779.

Antagonistic Activity of Bacterial Isolates

The antagonistic abilities of these bacterial isolates were determined against P. aphanidermatum using an in vitro dual-culture assay. The results indicated that none of the bacterial isolates showed considerable level of inhibition of mycelial growth of P. aphanidermatum. All the bacterial isolates recorded less than 5 mm inhibition zone (Table 2). Of the 6 bacterial isolates evaluated, S. epidermidis Sh3 produced the maximum inhibition zone of 4.2 mm.

Plant Growth Promoting Activity of Bacterial Isolates

The bacterial isolates were tested for plant growth promotion effects on cucumber using a roll paper towel

Table 2. Inhibition of mycelial growth of Pythium aphanidermatum by bacterial isolates from spent mushroom substrate of Agaricus bisporus

Bacterial Isolate	Inhibition zone (mm)
Staphylococcus epidermidis Sh1	3.0 ± 0.8^{abc}
Staphylococcus aureus Sh2	4.0 ± 0.8^{ab}
Staphylococcus epidermidis Sh3	4.2 ± 0.5^{a}
Bacillus albus Sh4	2.0 ± 0.8^{c}
Delftia lacustris Sh6	3.0 ± 0.0^{abc}
Comamonas aquatica Sh7	2.5 ± 0.6^{c}

Data are mean of four replications ± standard deviation.
Values in the column with the same letter are not significantly different from each other at P<0.05

technique. The results revealed that seed bacterization with C. aquatica Sh7, B. albus Sh4, D. lacustris Sh6 and S. epidermidis Sh3 resulted in a significant (F=9.57, df=6, p<0.05) increase in seedling vigour compared to control (Table 3). Among the various treatments, seeds treated with C. aquatica Sh7 showed the highest seedling vigor (Figure 1). No significant (p<0.05) difference in the % seed germination among the treatments was observed.

IAA Production

All the 6 isolates of bacteria tested produced IAA between 0.28±0.02 and 9.25±0.02 mg L^{-1} in tryptophan-amended growth medium (Table 4; Figure 2). The maximum (9.25 mg L^{-1}) and minimum (0.28 mg L^{-1}) production of IAA was recorded with C. aquatica Sh7 and S. epidermidis Sh1, respectively.

Discussion

The existence of a broad range of bacterial species in the SMS has been documented (Ntougias et al., 2004; Watabe et al., 2004). Ntougias et al. (2004) reported the presence of bacterial genera Arthrobacter, Brevibacterium, Bacillus, Comamonas, Carnobacterium, Desemzia, Microbacterium, Paenibacillus, Exiguobacterium, Sphingobacterium and Staphylococcus in the spent mushroom compost of Agaricus spp. By using DNA sequence typing, several bacterial species including, Bacillus subtilis, Bacillus licheniformis, Paenibacillus lentimorbus, Pseudomonas mevalonii, Stenotrophomonas sp., Klebsiella/Enterobacter sp., Microbacterium sp. and Sphingobacterium multivorum have been reported in the spent mushroom compost (Watabe et al., 2004). The type of substrates used in the compost preparation and their pasteurization conditions are known to influence the diversity of bacterial communities in SMS (Ntougias et al., 2004). Choudhary (2011) isolated Acinetobacter sp., Pseudomonas sp. and Sphingobacterium sp. from the casing material for Agaricus bisporus. Zhu et al. (2014) found Comamonas serinivorans sp. nov. in wheat straw compost. Silva et al. (2009) reported the presence of Bacillus, Paenibacillus spp. and Streptomyces in a sugarcane bagasse and Cynodon dactylon straw compost used for A. brasilienses cultivation. Gbolagade (2006) reported the presence of Pseudomonas aeruginosa, Enterobacter aerogenes, Micrococcus roseus, Bacillus subtilis, B. cereus, B. polymyxa, B. licheniformis, Escherichia coli, Clostridium perfringens and Citrobacter freundii in the compost used for cultivation of Lentinus squarrosulus and Pleurotus tuber-regium. In the present study, Staphylococcus epidermidis (Sh1 and Sh3), S. aureus (Sh2), Bacillus albus (Sh4), Delftia lacustris (Sh6) and Comamonas aquatica (Sh7) were detected in the SMS of A. bisporus. The primary source of these bacteria might be the casing material or compost or water used for cultivation of mushrooms (Rainey et al., 1990; Choudhary, 2011; Kertesz and Thai, 2018; Cao et al., 2019).

Several bacteria isolated from compost are reported to have ability to suppress the growth of plant pathogenic fungi (Boulter et al., 2002; Suarez-Estrella et al., 2007; Sreevidya and Gopalakrishnan, 2017) and to promote plant growth (Chin et al., 2017; Sreevidya and Go-

Table 3. Effect of bacterial isolates from spent mushroom substrate of Agaricus bisporus on cucumber seed germination and seedling vigor

Bacterial Isolate	% germination*	Shoot length (cm)	Root length (cm)	Vigour Index**
Staphylococcus epidermidis Sh1	75.0 ± 5.8	6.3 ± 1.5^{b}	14.8 ± 3.8^{ab}	1583 ± 293^{bc}
Staphylococcus aureus Sh2	72.5 ± 5.0	6.1 ± 1.5^{b}	16.1 ± 2.1^{a}	1612 ± 156^{bc}
Staphylococcus epidermidis Sh3	75.0 ± 5.8	7.0 ± 1.8^{b}	15.3 ± 1.9^{ab}	1671 ± 185^{b}
Bacillus albus Sh4	75.0 ± 5.8	8.0 ± 1.2^{ab}	16.6 ± 3.2^{a}	1844 ± 294^{ab}
Delftia lacustris Sh6	75.0 ± 5.8	8.0 ± 0.9^{ab}	16.1 ± 1.9^{a}	1805 ± 152^{ab}
Comamonas aquatica Sh7	77.5 ± 5.0	9.1 ± 1.6^{a}	17.0 ± 2.5^{a}	2018 ± 255^{a}
Control	72.5 ± 5.0	6.4 ± 1.5^{b}	12.1 ± 2.0^{b}	1343 ± 160^{c}

* Non-significant (P<0.05) . **Vigor index was calculated by multiplying the % germination of seeds with the sum of shoot length and root length. Data are mean of three replications ± standard deviation. Values in the column with the same letter are not significantly different from each other at P<0.05

Table 4. Production of IAA by bacterial isolates from spent mushroom substrate of Agaricus bisporus

Bacterial Isolate	IAA (mg L^{-1})
Staphylococcus epidermidis Sh1	0.28 ± 0.02^{e}
Staphylococcus aureus Sh2	1.07 ± 0.01^{c}
Staphylococcus epidermidis Sh3	0.77 ± 0.01^{d}
Bacillus albus Sh4	0.33 ± 0.00^{e}
Delftia lacustris Sh6	7.57 ± 0.07^{b}
Comamonas aquatica Sh7	9.25 ± 0.02^{a}

Data are mean of four replications ± standard deviation
Values in the column with the same letter are not significantly different from each other at $P<0.05$

palakrishnan, 2017). Riahi et al. (2012) identified three bacterial species viz, Bacillus subtilis, B. licheniformis and B. amyloliquefaciens from the extract of leached spent mushroom compost that showed antagonistic effect towards Lecanicillium fungicola, the causal agent of dry bubble disease of button mushroom. In the present study, none of the bacterial isolates showed substantial level of suppression of growth of P. aphanidermatum and all the bacterial isolates recorded less than 5 mm inhibition zone. However, plant growth promoting effect of these bacterial isolates was observed. Although no significant difference in % seed germination was observed, seed bacterization with C. aquatica Sh7, B. albus Sh4, D. lacustris Sh6 and S. epidermidis Sh3 resulted in a significant increase in seedling vigor of cucumber compared to control and C. aquatica Sh7 treated seeds showed the maximum seedling vigour. Several reports indicate the beneficial effects of bacteria present in the substrates used for cultivation of mushrooms (Rainey et al., 1990; Straatsma et al., 1994; Ahlawat and Vijay, 2010). The bacteria such as Alcaligenes faecalis and Pseudomonas putida which are surviving in casing layer are reported to influence the growth and morphogenesis of A. bisporus by producing growth inducing compounds, which stimulate initiation of pinheads (Rainey et al., 1990). Straatsma et al. (1994) demonstrated that the thermophilic fungi present in mushroom compost enhanced the growth rate of Agaricus mycelium up to two fold. Inoculation with Bacillus megaterium or Staphylococcus has been shown to enhance mushroom production and early cropping (Ahlawat and Vijay, 2010). The increase in seedling vigor of cucumber in the present study could be as a result of production and release of growth promoting compounds like IAA by the bacterial isolates.

IAA is a common auxin and is a product of L-tryptophan metabolism of microorganisms. In bacteria, IAA is primarily synthesized via the indole-3-pyruvic acid pathway (Gomes et al., 2017). IAA produced by plant growth-promoting rhizobacteria (PGPR) is known to enhance root growth (Persello-Cartieaux et al., 2003) and the growth of root hairs (Desbrosses et al., 2009). Asghar et al. (2002) observed a significant relationship between in vitro auxin production by PGPR and yield of Brassica juncea. Deepa et al. (2010) demonstrated that Enterobacter cloacae and Enterobacter aerogens strains, which produced IAA, exhibited growth-promoting effect in Vigna unguiculata. In addition to the effects of IAA produced by beneficial bacteria on plants, the growth and yield of mushrooms also reported to be influenced by IAA (Maniruzzaman et al., 2008; Ramachela and Sihlangu, 2016). Maniruzzaman et al. (2008) demonstrated that the culture media amended with IAA (5 ppm) caused rapid proliferation of oyster mushroom mycelia. Ramachela and Sihlangu (2016) reported that auxins promoted the cap size of Pleurotus ostreatus. In the present study, all the 6 bacterial isolates produced IAA in vitro and the production levels varied between 0.28 and 9.25 mg L^{-1}. Among the bacterial isolates tested, C. aquatica Sh7 showed the highest production of IAA (9.25 mg L^{-1}). The same bacterial isolate displayed the highest plant growth promoting activity. These results suggest that IAA produced by this bacterial isolate might have involved in enhancing vigor of cucumber seedlings. An interesting observation in our study is that the bacterial isolate B. albus B4, which is producing low amounts of IAA in vitro, enhanced the growth of cucumber. These results suggest that other mechanisms of action might have been involved in plant growth promotion by this bacterium. However, Schwachtje et al. (2012) reported that the non-growth promoting bacterial strains Pseudomonas sp. WCS417r and G53 isolated from the rhizosphere of Arabidopsis showed the highest levels of IAA production.

Conclusion

This study demonstrated the existence of different bacteria in SMS of Agaricus bisporus in Oman. These bacterial isolates displayed low levels of antagonism against P. aphanidermatum and produced less than 5 mm inhibition zone. However, these bacterial isolates enhanced the plant growth as demonstrated by increased seedling vigor of cucumber compared to control. The level of production of IAA by these bacterial isolates varied among isolates. Among the bacterial isolates tested, Comamonas aquatica Sh7 showed the highest production of IAA as well as plant growth promoting activity. Further studies are required to evaluate the potential of these bacterial isolates or their cell free culture filtrates in promoting growth of edible mushrooms and in enhancing plant growth under in vivo conditions.

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RESEARCH PAPER

Effect of Storage Time on the Quality of Smoked *Hetroclarias*

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تأثير وقت التخزين على جودة *Hetroclarias* المدخن

ايلوها و جيمو و عثمان و شيتو

ABSTRACT. In this study, the effect of storage time on the quality of smoked heteroclarias was studied. Samples (108) of heteroclarias (average weight 210 ± 15 g) were used. Proximate, mineral composition (Ca, Na, Fe and Mg), biochemical, amino acid and sensory characteristics were evaluated. Data obtained was subjected to Analysis of Variance (ANOVA), while the sensory data was subjected to nonparametric test (i.e. Kruskal Wallis test). Smoked heteroclarias was good nutritional quality in terms of compositions, such as protein, fat, carbohydrate, mineral and amino acids; however, these compositions were decreased with the increase of storage at ambient temperature. Glutamic acid was the most predominant amino acid and the highest non-essential amino acid (NEAA), while lysine was the most predominant essential amino acid (EAA). There was higher concentration of non-essential amino acids than essential amino acids, and EAA/NEAA ratio (0.86 – 0.93) indicated that the fish was excellent in terms of protein quality. Predicted protein efficiency ratio (PPER) ranged between 3.44-3.61 and its biological value ranged between 79.84-75.04. Chemical score and TEAA (Total Essential Amino Acid) decreased with the increase of storage time and its texture reduced significantly (χ^2 = 12.207, p≤0.01) with the increased storage period. Smoked heteroclarias could be recommended for the consumption owing to its retained nutritional quality.

KEYWORDS: Storage time; quality; smoked; heteroclarias.

المستخلص: تمت دراسة تأثير زمن التخزين على جودة *Hetroclarias* المدخنة. حيث تم استخدام عينات حوالي ١٠٨ من *Hetroclarias* (متوسط الوزن ٢١٠ ± ١٥ جم). تم تقييم الخصائص الكيميائية ، التركيب المعدني (Ca, Na, Fe and Mg) ، الكيمياء الحيوية ، الأحماض الأمينية والخصائص الحسية. خضعت البيانات التي تم الحصول عليها لتحليل التباين (ANOVA)،في حين تحليل البيانات الحسية باستخدام Kruskal Wallis test كانت *Hetroclarias* المدخنة ذات جودة غذائية جيدة من حيث التركيب الكيميائي ، مثل البروتين والدهون والكربوهيدرات والمعادن والأحماض الأمينية. ومع ذلك ، تقلصت كمية هذه التركيبات مع زيادة التخزين في درجة الحرارة المحيطة. كان حمض الجلوتاميك هو الأكثر شيوعًا من الأحماض الأمينية وأعلى الأحماض الأمينية غير الضرورية (NEAA)، بينما كان الليسين هو الأكثر شيوعًا من بين الأحماض الأمينية الأساسية (EAA) . وجد أن تركيز الأحماض الأمينية غير الضرورية أعلى من الأحماض الأمينية الأساسية ، وأشارت نسبة(٠,٨٦ - ٠,٩٣) EAA/NEAA إلى أن الأسماك كانت ممتازة من حيث جودة البروتين. تراوحت نسبة كفاءة البروتين المتوقعة بين ٣,٤٤-٣,٦١ وتراوحت قيمتها البيولوجية بين ٧٥,٠٤-٧٩,٨٤. انخفضت الدرجة الكيميائية وإجمالي الأحماض الأمينية الأساسية مع زيادة وقت التخزين وتناقص قوامه بشكل ملحوظ(χ^2 = ١٢,٢٠٧, p≥٠,٠١)، مع زيادة فترة التخزين. يمكن التوصية *Hetroclarias* المدخن للاستهلاك بسبب جودته الغذائية المحتفظ بها

الكلمات المفتاحية : وقت التخزين ، الجودة ، المدخن ، *Hetroclarias*

Introduction

Fish is one of the important source of protein, and it has high commercial and medicinal values due to the presence of essential amino acids, other nitrogenous compounds, water, lipids, carbohydrates, minerals and vitamins (Marwa, 2015; Ayeloja et al., 2011a). Zulema (2014) recommended the consumption of fish as it prevents cardiovascular and other diseases. Ravichandran et al. (2011) also reported that fish is a good source of antimicrobial peptides, which defend the body against dreadful human pathogens. Fish also contributes to income, employment generation and foreign exchange earning of many countries (Zulema, 2014). Fish contains all the essential amino acids, hence it is called "complete protein"; thereby making its consumption a necessity (Pawar and Sonawane, 2013). Heliene (2016) also stated that fish have high levels of polyunsaturated fatty acids that are important for the promotion and maintenance of health as well as minerals, such as calcium, phosphorus, sodium, potassium and magnesium. However, fish is highly perishable and considerable losses in quality could occur before consumption if not properly handled, processed and stored. Therefore, it is a concern for the fisheries industry all over the world (Huss et al., 2004). Fresh fish deteriorates very quickly after harvesting due to the actions of enzymes and bacteria (Akande, 1996). Fish quality is a complex concept involving a number of factors (Jinadasa, 2014).

To reduce fish spoilage, various preservation and processing methods are employed including freezing, chemical preservation, salting, smoking and frying (Ayeloja et al., 2018). However, smoking is one of the

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most popular method of fish processing in developing countries like Nigeria (Ayeloja, 2019). Smoking provides good taste and aroma to fish and it extends fish shelf life. This is due to the effects of dehydration, antimicrobial and antioxidant activities of several components from smoke, such as formaldehyde, carboxylic acids and phenols (Serkan et al., 2010). However, there is limited information on the effect of storage time on the quality of smoked heteroclarias, which is one of the most cultured fish species. Heteroclarias is more cultured due to its superior growth, improved survival and general hardiness than culturing the pure breed of either Clarias gariepinus or Heterobranchus bidorsalis (Obe, 2014). Owodehinde et al. (2018) reported that heterobranchus is a hybrid product of H. bidorsalis (♂) x C. gariepinus (♀) which are fresh water fish. Heteroclarais is produced from the two species due to their uniqueness and prominence among commercial fish farmers in Africa and these fishes are tasty, hardy and tolerant to poor quality of growing water (Ekelemu, 2010). However, consumers rarely have information about the nutritional quality of smoked heteroclarias and its quality changes during storage, thus the need for this study. This study aimed to determine the nutritional quality of smoked heteroclarias as well as to examine the effect of ambient storage on its proximate, mineral, biochemical, amino acid and sensory qualities.

Materials and Methods

Sample Collection

Total 108 samples of heteroclarias (average weight 210 + 15g) were collected at a commercial fish farm within Ilorin metropolies, Kwara state, North-Central Nigeria. These were taken to laboratory; and were gutted, washed and smoked similar to the modified method of Ayeloja et al. (2015) (Figure 1). The smoking was performed using NIOMR (Nigeria Institute of Oceanography and Marine Research) smoking kiln, which was manually powered using charcoal as fuel. The smoked fish was stored at ambient temperature (27 ± 3°C) and samples were collected at every fortnight (i.e., 0, 14, 28, 42 and 56 days) for proximate, mineral, biochemical; and samples for organoleptic assessment and amino acid analysis were collected at day 0, 28 and 56, respectively.

Composition Analysis

Proximate compositions of fish were determined by conventional method (AOAC, 2000). Petri dish was cleaned and weighed. Then 1.0 g of each of the grounded fish samples was measured in each petridish and then weighed. They were each transferred into the oven at 105°C for 3 hours. After the first 3 hours, the petridish was removed from the oven, allowed to cool and weighed. The petridish was returned into the oven and was brought out after an hour and weighed again; this process was repeated until a constant weight was achieved and moisture content was determined.

The crude protein was determined using Kjeldahl method. The fish sample (either smoked or frozen) was ground into a fine or smooth texture. A known weight (5.0 g) of the fish sample is then weighed into a long necked Kjedahl flask along with 5 g of copper sulphate anhydrous and 5 g of sodium sulphate anhydrous. Then, 25 ml of concentrated sulphuric acid (H_2SO_4) was added. The flask was gently placed and the content was heated, the heating continued until a clear solution was obtained. The digestion was performed between 3 to 5 hours. The clear hot solution obtained was allowed to cool and solution was filtered using filter paper. Then, 5 ml of the filtered digested sample was poured into the protein determination equipment and 10 ml of 40% NaOH was added followed by a distillation process. The steam being passed in the reactor condenses and drops into a conical flask containing boric acid (5 ml) until the mixture changes color. After changing color, 50 ml of the liquid was collected and titrated with 0.01 M of HCl until the color (green) changed to deep blue.

For the estimation of fat content, the dried samples left after moisture determination were finely ground and the fat was extracted for 4 hours with a non-polar solvent (i.e. ethyl ether) using soxhlet extraction method. After extraction, the solvent was evaporated and the extracted fat was weighed. Ash was determined by burning the dried sample in a furnace at 550°C for 4 h. The difference in weights before and after burning gave the total ash content. The total carbohydrate content was determined by subtracting the sum of the percentage moisture, ash, crude lipid, and crude protein from 100%.

Mineral Composition and other Biochemical Test

Crucible was cleaned, weighed and then 5.0 g of ground fish sample was measured into each crucible. This was transferred into the oven at 60°C for 45 minutes to 1 hour. After oven drying, the sample was weighed into a conical flask and was digested using nitric acid and hydrochloric acid. After digestion, the concentration of the minerals was determined using Pinnacle 900T Atomic Absorption Spectrophotometer (AAS). The Total Volatile Base Nitrogen (TVBN), trimethyl amine (TMA), pH, peroxide value (PV) and free fatty acid (FFA) were determined following the method of Pearson (1982).

Amino Acid Analysis

The preparation of the fish samples was adapted from the procedure described by Benitez (1989). The fish samples were dried to a constant weight, defatted, and hydrolyzed (Bligh and Dyer, 1959). These were evaporated in a rotary evaporator and loaded into the Applied Biosystems PTH Amino Acid Analyzer Model 120A.

Hydrolysis

A known weight (2.0 g) of the defatted sample was weighed into a glass ampoule and 7 ml of 6 N HCl was added. In order to avoid possible oxidation of some amino acids during hydrolysis such as methionine and cystine, nitrogen was passed into the ampoule to expel oxygen. The glass ampoule was then sealed with Bunsen burner flame and placed in an oven preset at 105±5°C for 22 hours. The ampoule was allowed to cool before broken open at the tip and the content was filtered to remove the humins. The filtrate was then evaporated to dryness at 40°C under vacuum by a rotary evaporator. The residue was dissolved with 5 ml to acetate buffer (pH 2.0) and stored in plastic specimen bottles, which were kept in a freezer. It is noteworthy that hydrolysis procedure was unable to determine tryptophan since it is chemically decomposed by 6N HCl during acid hydrolysis.

Tryptophan

To identify tryptophan, a separate sample of the defatted sample was hydrolysed using antioxidants such as dodecanethiol to replace 6 N hydrochloric acid (HCl), thereby preserving tryptophan. The tryptophan in the known sample was hydrolyzed with 4.2 M Sodium hydroxide (Maria et al., 2004). The known sample was dried to constant weight, defatted and hydrolyzed; and defatted sample (2.0 g) was weighed into glass ampoule. It is recommended that alkaline hydrolysis produced higher tryptophan recovery than acid hydrolysis. Sodium hydroxide was used instead of barium hydroxide to avoid problems of precipitation and adsorption of tryptophan (Maria et al., 2004). Nitrogen was passed into the ampoule to expel oxygen and it was then sealed with Bunsen burner flame. The sealed ample was placed in an oven preset at 105±5°C for 4 hours. The ampoule was allowed to cool and the content was filtered to remove the humins. The filtrate was then neutralized to pH 7.0 and evaporated to dryness at 40°C using a rotary evaporator under vacuum. The residue was dissolved in 5 ml of borate buffer (pH 9.0) and stored in plastic specimen bottles, which were kept in a freezer.

Loading of Hydrolysate into a PTH analyser

Sixty microliter of the hydrolysate was loaded in the analyzer. This was dispensed into the cartridge of the analyzer. The analyzer is designed to separate and analyze free acidic, neutral and basic amino acids. The period of the analysis lasted for 45 minutes. To calculate amino acid values, an integrator attached to analyzer calculated the peak area proportional to the concentration of each of the amino acid. The net height of each peak produced by the chart recorder of TSM (each representing an amino acid) was measured. The half-height of the peak on the chart was found and the width of the peak on the half-height was accurately measured and recorded. Approximate area of each peak was then obtained by multiplying the height with the width at half-height. The norleucine equivalent (NE) for each amino acid in the standard mixture was calculated using the formula:

$$\text{Norleucine (NE)} = \frac{\text{Area of norleucine peak}}{\text{Area of each amino acid}} \quad (1)$$

where, NE is an internal standard. A constant S was calculated in g/100g protein using the following formula:

$$S_{std} = NE_{std} \times \text{Mol. Weight} \times \mu MAA_{std} \quad (2)$$

Finally, the amount of each amino acid present in the sample was calculated in g/100 g protein using the formula:

$$\text{Concentration} \left(\frac{g}{100g}\text{protein}\right) = NW \times W@\ NH/2 \times Sstd \times C \quad (3)$$

$$C = Dilution \times \frac{16}{Sample} wt\ (g) \times N\% \times 10. Vol\ Loaded \div NH \times W(nleu) \quad (4)$$

where NH is net height, W is width @ half height, nleu = Norleucine. The period of analysis lasted for 45 minutes. To determine nitrogen in the separated sample for analyzing tryptophan, a 200 mg ground sample was weighed, wrapped in whatman filter paper (No. 1) and the procedure for nitrogen determination for amino acids as described was repeated. Percentage nitrogen was calculated.

Predicted Protein Efficiency Ratio (PPER)

The predicted protein efficiency ratio (PPER) was estimated by using the equation given by Alsmeyer et al. (1974):

$$PPER = -0.468 + 0.454(Leu) - 0.105(Tyr). \quad (5)$$

Amino Acid Score (AAS)

The essential amino acid score was calculated based on the whole hen's egg amino acid profiles (Paul and Southgate, 1976)

$$\text{Amino acid score} = \frac{\text{Amount of amino acid per test protein (g/100 g)}}{\text{Amount of amino acid per protein in reference (g/100 g)}} \quad (6)$$

Essential Amino Acid Index (EAAI)

The essential amino acid index (EAAI) was calculated using the ratio of test protein to the reference protein for each ten essential amino acids (Oser, 1959) as:

$$EAAI = \sqrt[n]{\frac{\text{lysine P}}{\text{Lysine S}} \times \frac{\text{tryptophan P}}{\text{tryptophan S}} \times \ldots \times \frac{\text{threonine P}}{\text{threonine S}}} \quad (7)$$

where, P is test protein, and S is standard whole egg protein.

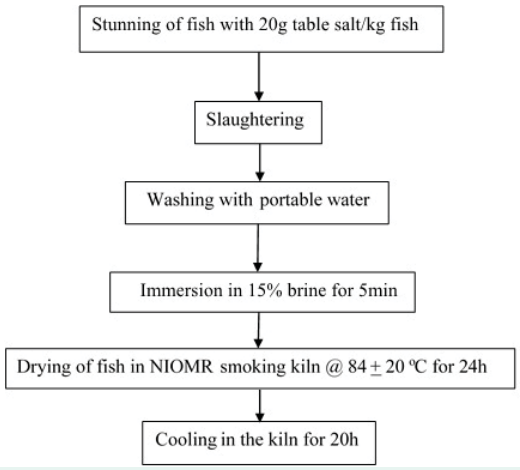

Figure 1. Flow chart for the production of smoked catfish, heteroclarias

Biological Value (BV)

The Biological Value (BV) was calculated by the method of Oser (1959): BV= 1.09 (EAAI) - 11.73 (8)

Organoleptic Assessment

The various smoked fish species were subjected to consumer preference evaluation using based on 5-point hedonic scale modified from Tobor (1994) and Eyo (2001). Odor, flavor 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. Thirty semi-trained panelists from Department of Aquaculture and Fisheries, Faculty of Agriculture University of Ilorin Kwara State, Nigeria were used for the assessment.

Statistical Analysis

SPSS 16.0 version was used for the statistical analysis. Data collected on descriptive organoleptic assessment using hedonic scale were subjected to nonparametric test (Kruskal Wallis test). While other data were subjected to Analysis of variance (ANOVA) using F-test to determine the significant difference between the treatments. Means of the significantly different treatments were separated using Duncan multiple range test at 95% confidence value. Samples for laboratory analysis were replicated thrice to aid statistical analysis.

Results

The result for the proximate composition of smoked Heteroclarias is presented on Table 1. The result indicates that the moisture content of smoked Heteroclarias ranged from 26.44 ± 0.01 to 27.37 g/100 g sample ± 0.06 within a storage time of 56 days. The highest moisture content (27.37 g/100 g sample ± 0.06) was recorded on the 56th day of storage, while the lowest moisture content 26.44 g/100 g sample ± 0.01 was recorded on day 0; which was immediately after smoking. The moisture content of the fish increased significantly (p≤0.05) with the increased storage time through the period of the storage (56 days).The percentage crude protein content of the fish ranged from 45.37 g/100 g sample ± 0.15 to 48.96 g/100 g sample ± 0.03 within a storage time of 56 days. There was no significant difference (p>0.05) in the crude protein content for the first 14 days of storage but later significantly increased (p≤0.05) from day 28 until storage period. The highest crude protein value of 48.96 g/100 g sample ± 0.03 was recorded on the day 0 before fish was stored at ambient, while the lowest value of 45.37 g/100 g sample ± 0.15 was recorded on the 56th day of storage.

The crude lipid content of the fish ranged between 18.73 ± 0.12 to 20.42 g/100 g sample ± 0.02 within a storage period of 56 days. The highest crude lipid content (20.42 g/100 g sample ± 0.02) was recorded at day 0, which was the lowest crude lipid content (18.73 g/100 g sample ± 0.12) was recorded on the 56th day of storage. The lipid content of the fish decreased significantly (p≤0.05) with the increased storage time through the storage period (56 days). The ash content of the fish differed significantly (p≤0.05) during the first 14 days of storage. However, there was no significant difference (p>0.05) in the ash content of the fish from day 28 until the remaining period of storage. The percentage carbohydrate content of smoked Heteroclarias ranged between 2.02 ± 0.01 to 6.38 g/100 g sample ± 0.27 during the storage period of 56 days. No significant difference (p>0.05) was observed for the first 2 weeks of storage but later significantly differed (p≤0.05) from 28 days un-

Table 1. Proximate composition (g/100g sample) of smoked Heteroclarias with increased storage time

	Day 0	Day 14	Day 28	Day 42	Day 56
Moisture	26.44 ± 0.01^{a}	26.78 ± 0.02^{b}	26.91 ± 0.08^{c}	27.17 ± 0.05^{d}	27.37 ± 0.06^{e}
Crude protein	48.96 ± 0.03^{d}	48.96 ± 0.02^{d}	46.86 ± 0.08^{c}	45.91 ± 0.08^{b}	45.37 ± 0.15^{a}
Crude lipid	20.42 ± 0.02^{d}	20.02 ± 0.01^{c}	19.00 ± 0.20^{b}	18.94 ± 0.12^{ab}	18.73 ± 0.12^{a}
Ash	2.16 ± 0.02^{a}	2.24 ± 0.01^{b}	2.16 ± 0.03^{a}	2.16 ± 0.03^{a}	2.15 ± 0.02^{a}
CHO (Carbohydrate)	2.02 ± 0.01^{a}	2.01 ± 0.01^{a}	5.07 ± 0.16^{b}	5.82 ± 0.21^{c}	6.38 ± 0.27^{d}

*Mean with different superscript in the row indicates significant difference at p< 0.05

Table 2. Mineral composition of smoked Heteroclarias (mg/100 g ash) with increased storage time

Mineral	Day 0	Day 14	Day 28	Day 42	Day 56
Calcium (Ca)	6.15 ± 0.07^{d}	5.85 ± 0.07^{c}	5.60 ± 0.00^{b}	5.44 ± 0.06^{a}	5.33 ± 0.04^{a}
Sodium (Na)	1.05 ± 0.07^{b}	0.95 ± 0.07^{b}	0.81 ± 0.01^{a}	0.77 ± 0.02^{a}	0.70 ± 0.01^{a}
Iron (Fe)	0.41 ± 0.01^{d}	0.39 ± 0.01^{d}	0.34 ± 0.02^{c}	0.28 ± 0.03^{b}	0.21 ± 0.01^{a}
Magnesium (Mg)	4.35 ± 0.21^{d}	3.95 ± 0.07^{c}	3.45 ± 0.07^{b}	3.25 ± 0.07^{ab}	3.00 ± 0.00^{a}

*Mean with different superscript in the row indicates significant difference at $p<0.05$

Table 3. Effect of storage time on biochemical quality of smoked Heteroclarias.

	TMA (mgN/100 g)	TVBN (mgN/100 g)	pH	PV (mEq. Peroxide/kg)	FFA (g/100 g fat)
Day 0	$21.02+0.01^{a}$	$30.37+0.01^{a}$	$7.43+0.06^{a}$	$8.47+0.01^{b}$	$7.83+0.02^{c}$
Day 14	$22.50+0.02^{b}$	$31.50+0.01^{c}$	$7.57+0.06^{c}$	$8.70+0.01^{e}$	$7.41+0.01^{b}$
Day 28	$24.09+0.11^{c}$	$32.86+0.02^{d}$	$7.43+0.06^{d}$	$8.65+0.01^{d}$	$7.38+0.03^{b}$
Day 42	$24.16+0.02^{c}$	$32.73+0.56^{d}$	$7.63+0.06^{d}$	$8.61+0.01^{c}$	$7.40+0.00^{b}$
Day 56	$21.08+1.14^{a}$	$30.95+0.07^{b}$	$7.63+0.06^{b}$	$8..22+0.01^{a}$	$6.92+0.00^{a}$

Means ± SD with different superscript in the same column indicating significant differences at $P \leq 0.05$.
Note: TMA= Trimethylamine, TVBN= Total Volatile Base Nitrogen, PV= Peroxide Value, FFA= Free Fatty Acid.

til the remaining 56 days of storage.

Table 3 presents the effect of storage time on biochemical quality of smoked Heteroclarias. The highest TMA value (24.16 ± 0.02 mgN/100 g) and the lowest value (21.02 ± 0.01 mgN/100 g) was recorded with values increasing significantly ($P \leq 0.05$) from day 0 to day 42 of storage time and decreased significantly ($P>0.05$) from day 42 to day 56 of storage time. The highest TVBN value (32.86 ± 0.02 mgN/100 g) and the lowest (30.37 ± 0.01 mgN/100g) was recorded. It was observed that the TVBN increased significantly ($P \leq 0.05$) from day 0 to day 28 of storage time and decreased significantly ($P>0.05$) from day 28 to day 56. The pH had no significant differences ($P>0.05$) with increased in storage time. The PV had no significant differences ($P>0.05$) with the increase in storage time. The highest FFA (7.83 g/100 g fat ± 0.02) and the lowest value (6.92 g/100 g fat ± 0.00) was observed. However, FFA decreased significantly ($P>0.05$) from day 0 to day 28 and slightly increased significantly at day 42 but decreased significantly from day 42 to day 56 of storage time.

Amino Acid Profile

The amino acid profile of Heteroclarias spp. muscles at different storage time (day 0, day 28 and day 56) are presented in Table 4. Eighteen amino acids (10 essential, 8 non-essential) were observed in the fish with their mean values as shown in the Table 4. The highest and lowest mean value at day 0 was observed in glutamic acid (14.38 g/100 g crude protein, cp) and tryptophan (0.84 g/100 g cp) respectively. The highest and lowest mean value at day 28 was observed in glutamic acid (13.36 g/100 g cp) and tryptophan (0.87 g/100 g cp), respectively. The highest and lowest mean value at day 56 was observed in glutamic acid (14.91 g/100 g cp) and cystine (0.61 g/100 g cp), respectively. An increase in amino acid content was observed across storage time (i.e. as storage time increases) in leucine (from 7.28 ± 0.10 to 7.36 ± 0.08 to 7.59 ± 0.29 g/100 g cp) and aspartic acid (from 9.91 ± 0.02 to 10.00 ± 0.03 to 9.97 ± 0.11 g/100 g cp), which increased at day 28 but decreased slightly at day 56. Decrease content was observed across storage time in valine (from 5.00 ± 0.04 to 4.65 ± 0.08 to 3.74 ± 0.08 g/100 g cp), methionine (from 2.94 ± 0.08 to 2.33 ± 0.10 to 1.74 ± 0.04 g/100 g cp), glycine (from 8.18 ± 0.18 to 8.15 ± 0.17 to 5.25 ± 0.10 g/100g cp), tyrosine (from 3.19 ± 0.12 to 2.93 ± 0.25 to 2.92 ± 0.11 g/100 g cp) and serine (from 4.81 ± 0.08 to 4.62 ± 0.11 to 3.77 ± 0.10 g/100 g cp). In phenylalanine (from 4.43 ± 0.00 to 3.72 ± 0.00 g/100 g cp), tryptophan (from 0.87 ± 0.04 to 1.73 ± 0.00 g/100g cp), proline (from 6.14 ± 0.21 to 4.42 ± 0.08 g/100 g cp), arginine (from 6.88 ± 0.25 to 6.20 ± 0.1 g/100 g cp), histidine (from 2.27 ± 0.04 to 2.09 ± 0.11 g/100 g cp), cystine (from 0.97 ± 0.00 to 0.61 ± 0.00 g/100 g cp) and alanine (from 6.43 ± 0.08 to 5.80 ± 0.06 g/100 g cp), an increase was observed at day 28, followed by a decreased at 56 days after smoking. Decrease was observed at day 28 with an increase at day 56 in lysine (from 8.54 ± 0.00 to 8.51 ± 0.04 to 8.91 ± 0.49 g/100g cp), isoleucine (from 4.23 ± 0.05 to 3.11 ± 0.62 to 4.01 ± 0.07 g/100g cp), glutamic acid (from 14.38 ± 0.21 to 13.36 ± 0.06 to 14.91 ± 0.11 g/100 g cp) and threonine (from 4.36 ± 0.04 to 3.80 ± 0.20 to 5.00 ± 0.01 g/100 g cp). In one-way ANOVA test of the samples, no significant difference ($p>0.05$) was observed in leucine, lysine,

Table 4. Changes in amino acid profile (g/100 g cp) of hybrid catfish (Heteroclarias spp) across storage time

Amino acids	Day 0	Day 28	Day 56
Essential (g/100 g protein)			
Leucine	7.28±0.10^{a}	7.36±0.08^{a}	7.59±0.29^{a}
Lysine	8.54±0.00^{a}	8.51±0.04^{a}	8.91±0.49^{a}
Isoleucine	4.23±0.05^{a}	3.11±0.62^{a}	4.01±0.07^{a}
Phenylalanine	4.00±0.14^{b}	4.43±0.00^{a}	3.72±0.00^{c}
Tryptophan	0.84±0.05^{b}	0.87±0.04^{b}	1.73±0.00^{a}
Valine	5.00±0.04^{a}	4.65±0.08^{b}	3.74±0.08^{c}
Methionine	2.94±0.08^{a}	2.33±0.10^{b}	1.74±0.04^{c}
Arginine	6.67±0.06^{ab}	6.88±0.25^{a}	6.20±0.12^{b}
Threonine	4.36±0.04^{b}	3.80±0.20^{c}	5.00±0.01^{a}
Histidine	2.19±0.02^{a}	2.27±0.04^{a}	2.09±0.11^{a}
Non Essential (g/100g)			
Cystine	0.85±0.00	0.97±0.00	0.61±0.00
Alanine	6.41±0.16^{a}	6.43±0.08^{a}	5.80±0.06^{b}
Glutamic acid	14.38±0.21^{b}	13.36±0.06^{c}	14.91±0.11^{a}
Glycine	8.18±0.18^{a}	8.15±0.17^{a}	5.25±0.10^{b}
Serine	4.81±0.08^{a}	4.62±0.11^{a}	3.77±0.10^{b}
Aspartic acid	9.91±0.02^{a}	10.00±0.03^{a}	9.97±0.11^{a}
Proline	5.69±0.44^{a}	6.14±0.21^{a}	4.42±0.08^{b}
Tyrosine	3.19±0.12^{a}	2.93±0.25^{a}	2.92±0.00^{a}
	2.93±0.25^{a}	2.92±0.00^{a}	

Means ± SD with different superscript in the same column indicating significant differences at P ≤ 0.05.

isoleucine, cystine, tyrosine, aspartic acid and histidine content of the sample throughout the storage period. A significant difference (p≤0.05) was noticed in phenylalanine, threonine, glutamic acid, valine and methionine content of the sample at all storage times. No significant difference was observed in arginine between the periods of 0-28 days and 0-56 days of storage (p>0.05), however a significant difference was noticed between 28 and 56 days of storage (p≤0.05). Alanine, tryptophan, proline, glycine and serine showed similar trend of no significant difference (p>0.05) at day 0 and 28 days, but a statistical variation (p≤0.05) was noted at 56 days of storage.

Table 5 shows the protein quality parameters of Heteroclarias as a function of storage time. A decrease in total amino acid was observed with the increased storage time. A higher concentration of total non-essential amino acid was recorded than total essential amino acid. The table also records the ratio of EAA to NEAA, which was 0.86 at the beginning of the storage period, decreased slightly at day 28 to 0.84 and slightly increased at

Table 5. Changes in quality parameters of amino acid profile of Heteroclarias with increased storage time.

Protein Quality Parameters	Day 0	Day 28	Day 56
TAA (g/100g)	99.47	96.81	92.38
TEAA (g/100g)	46.05	44.21	44.33
TNEAA (g/100g)	53.42	52.60	47.65
EAA/NEAA	0.86	0.84	0.93
CS (g/100g)	53.33	51.67	50.13
EAAI	0.84	0.80	0.83
BV	79.84	75.04	78.36
PPER (g/100g)	3.44	3.50	3.61

Note: TAA= total amino acids; TEAA= total essential amino acid; TNEAA= total non-essential amino acid; EAAI = essential amino acid index; CS= chemical score; BV= biological value; PPER= predicted protein efficiency ratio.

day 56 to 0.93. Tryptophan was recorded as the limiting amino acid in the sample with chemical scores of 0.47 and 0.48 at day 0 and day 28 respectively while at day 56, valine was recorded as the limiting amino acid in the sample with chemical scores of 0.50. The highest EAAI value of 0.84 was recorded at day 1 and decreased to 0.80 and increased slightly to 0.83 at day 28 and day 56, respectively. The predicted protein efficiency ratio (PPER) ranged between 3.44 to 3.61 g/100 g cp. The highest and least biological values were recorded in day 0 and day 28, respectively.

Table 6 indicates the amino acid scores of the fish in relation to the amino acid scoring pattern of whole hen's egg protein. The values were found to be favorable in fish sample at day 0 of smoking and decreased at the end of storage period. Lysine had the highest amino acid score ranging from 1.37 to 1.38 g/100 g cp. Tryptophan and valine were observed as the limiting amino acid with values ranging between 0.47 to 0.96 g/100 g cp and 0.50 to 0.67 g/100 g cp, respectively.

In Table 7, the taste panelist scores allotted for texture of smoked Heteroclarias reduced significantly (χ^2 = 12.207, p≤0.01) with increased storage period, whereas no significant (χ^2= 1.628, 8.982, p>0.05) decrease in the physical quality of odor and flavor was observed during storage period.

Discussion

The result of the proximate composition of smoked Heteroclarias (Table 1) was similar to that reported for other smoked fresh water fishes (Ayeloja et al., 2011a; Abraha et al., 2018). The result indicated that crude protein varied within 48.96 and 45.3 g/100 g sample) and it reduced with the increase of storage time. The reduction of lipid was observed, while the moisture and carbohydrate were increased with the increase of storage time. Sim-

Table 6. Amino acid scores and Indispensable Amino acid index (IAAI) of Heteroclarias

Essential amino acids	Amino acid scores (g/100g)			Whole egg protein (g/100 g sample)	FAO/WHO provisional amino acid scoring pattern (g/100g)
	Day 1	Day 28	Day 56		
Leucine	0.88	0.89	0.91	8.3	7.0
Lysine	1.38	1.37	1.37	6.2	5.5
Isoleucine	0.76	0.56	0.72	5.6	4.0
Phenylalanine	0.78	0.87	0.73	5.1	+tyr 6.0
Tryptophan	0.47	0.48	0.96	1.8	1.0
Valine	0.67	0.62	0.50	1.5	5.0
Methionine	0.92	0.73	0.54	3.2	+cys 3.5
Arginine	1.09	1.13	1.02	6.1	
Histidine	0.83	0.91	0.95	2.4	
Threonine	0.85	0.75	0.98	5.1	4.0
EAAI	0.84	0.80	0.83		

EAAI = essential amino acid index; Whole hen's egg protein: adopted from Paul and Southgate (1976); FAO/WHO provisional amino acid scoring pattern: FAO/WHO (1973)

ilar results were reported by Ayeloja et al., 2011b and Mosarrat et al. (2016). They mentioned these changes could induce the gradual degradation of initial crude protein to more volatile products, such as total volatile bases, hydrogen sulphide and ammonia. Table 2 shows that Ca (6.15-5.33 mg/100 g ash) was the most abundant mineral in smoked heteroclaraias followed by Mg (4.35-3.00 mg/100 g ash). Adeyemi et al. (2013a) observed similar result in the case of Trachurus trachurus. They reported that calcium was the most abundant mineral in raw and smoked Trachurus trachurus. Table 3 shows that the TMA, TVBN, pH and PV of smoked heterobranchus increased significantly (P>0.05) with increase storage time until 42days, while the FFA decreased with the increase in storage time. Khanipour and Mirzakhani (2013) reported that the pH, PV and TVB-N values of hot smoked rainbow trout (Oncorhynchus mykiss) increased with increasing storage time. Mosarrat et al. (2016) also reported an increase of TVBN of smoked Chapila (Hamilton-Buchanan, 1822), Kaika (Hamilton-Buchanan, 1822) and Baim (Hamilton-Buchanan, 1822) when stored at ambient temperature, which was attributed to the formation of volatile amine compounds by autolytic process. Adeyemi et al. (2013b) also gave similar report that the TVBN of smoked Trachurus trachurus increased from 28.12 ± 0.38 mg N/100 g to 31.90 ± 0.3 mgN/100 g during storage. The recommended limit of acceptability of TVBN for fish is 20-30 mg N per 100 g (Daramola et al., 2007), while Kirk and Sawyer suggested a value of 30 – 40 mg N/100 g as the upper limits. The results in this study was observed within these values. The values recorded for PV in this study (8.22 – 8.70) is lower than those reported by Adeyeye et al. (2015) for smoked fishes sold in Lagos Nigeria (9.05 - 9.35 eroxide/kgrespectively). Eighteen amino acids (10 essentials, 8 non-essentials) were observed in heteroclarias (Table 4). Glutamic acid had the highest concentration among the amino acid and the non-essential amino acids while tryptophan was the least concentrated. While lysine was the most concentrated essential amino acid and tryptophan was the least concentrated essential amino acid in the fish. The amino acids reduced significantly ($p<0.05$) with the increase in storage time. Shiming et al. (2013) equally observed that glutamic acid was the most predominant amino acid and the non-essential amino acid in yellowfin tuna (Thunnus Albacares) and big eye tuna (Thunnus Obesus), while the lysine was the most predominant essential amino acid. This study established that heteroclarias contained amino acid as required by human. The glycine is major component of human skin collagen, together with other amino acids such as alanine, proline, arginine, serine, isoleucine, and phenylalanine form polypeptides (Zhao et al., 2010). A decrease in protein quality parameters of Heteroclarias with the increase in storage time was observed in this study (Table 5). A higher concentration of total non-essential amino acid was recorded than total essential amino acid. EAA to NEAA ratio is an index to define the quality of the protein (ElShehawy et al., 2016). The ratio of EAA to NEAA in this study ranged between 0.86 and 0.93 which was higher than 0.71 as reported for gilt head sea bream (Sparus aurata) and 0.65 reported for sea urchin roe (Pinto, 2007). The chemical score and TEAA decreased with the increase in storage time, the EAAI ranged between 0.84 and 0.80. The predicted protein efficiency ratio (PPER) ranged between 3.44 to 3.61 g/100 g cp was higher than 2.22 as recorded for C.

Table 7. Effect of storage time on organoleptic quality (i.e. consumer preference) of smoked Heteroclarias

	Day 0	Day 14	Day 28	Day 42	Day 56	χ^2	P-value
Odor	8.27	7.80	8.00	7.73	8.00	1.63	0.80
Flavor	8.87	7.90	8.07	7.93	8.73	8.98	0.06
Texture	9.33	8.00	8.00	7.93	8.33	12.21*	0.02

Kruskal Wallis test (χ^2) is significant along the row P≤0.05.

anguillaris and 1.92 recorded for O. niloticus (Adeyeye et al. 2009). The biological value of heteroclarias ranged between 79.84 to 75.04. Table 6 shows that the amino acid scored as compared to egg protein. The values obtained for smoked heteroclarias were found to be far lower than that of whole egg and it decreased with the increase of storage period. Lysine had the highest amino acid score ranging from 1.37 to 1.38 g/100 g cp, while the values for tryptophan and valine were observed to be very low ranging between 0.47 to 0.96 g/100 g cp and 0.50 to 0.67 g/100g cp, respectively. The quality of the texture of smoked heteroclarias (Table 7) reduced significantly (χ^2 = 12.207, p≤0.01) with increased storage period. However, the taste panel observed no significant (χ^2 = 1.628, 8.982, p>0.05) decrease in the odor and flavor of the fish during the storage period.

Conclusion

This study established that smoked heteroclarias had good nutritional quality in terms of compositions, which decreased with the increase of storage at ambient temperature. Minerals were abundant with highest Ca followed by Mg. Glutamic acid was the most predominant amino acid and the non-essential amino acids and tryptophan was the least concentrated; while lysine was the most predominant essential amino acid and tryptophan was the least concentrated essential amino acid in the fish. This study established that heteroclarias could provide good concentration of amino acid as require for human. The ratio of EAA to NEAA ranged between 0.86 and 0.93 and the predicted protein efficiency ratio (PPER) obtained in this study was higher than many other fish species. The biological value of heteroclarias ranged between 79.84 and 75.04. Its chemical score and TEAA decreased with increase in storage time. However, its texture quality reduced significantly (χ^2 = 12.207, p≤0.01) with increased storage period.

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RESEARCH PAPER

Farmers' Perceptions on the Influence of Inter-Communal Conflicts on the Agricultural Land in Share/Tsaragi Communities, Kwara State, Nigeria

تصورات المزارعين عن تأثير النزاعات الطائفية على الأراضي الزراعية في مجتمعات شير/ تساراجي ، ولاية كوارا ، نيجيريا

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ABSTRACT. Inter-communal conflicts have plagued rural communities which produce the bulk of agricultural produce in Nigeria. One such conflict is Share/Tsaragi inter-communal conflict. The objective of the study was to assess perceptions of farmers on the influence of inter-communal conflicts on the agricultural production in Share and Tsaragi communities in Edu and Ifelodun Local Government Areas (LGAs) of Kwara State, Nigeria. A three-stage random sampling procedure was used to select 120 respondents for the study. Data were collected using an interview schedule technique. A Likert-type five-point continuum scale was used to rank farmers' perceptions with five (5) as the most and one (1) as the worst check. The results showed that 68% of the respondents were above forty (40) years of age and 70% have resided in the communities for more than ten (10) years. Respondents perceived that conflict of resource which bordered on the boundary issue, claim of ownership of fertile land at Fejewe area, conversion of leased land to personal property among others are causes of the conflicts within the two communities. The results equally revealed the perceptions of respondents on the effects of conflicts in the area to include scarcity of arable land, displacement of farmers, destruction of lands and crops, scarcity of food and destruction of stored produce among others. The study showed the relationship between selected socio-economic characteristics and perception. This study concludes that there should be a clear delineation of the boundary within the two communities, the lift of the ban on some arable lands seized by the government and the promotion of other secondary occupations to relieve pressure on the land.

KEYWORDS: Conflict; Farmers; Perception; Kwara State; Share; Tsaragi.

المستخلص:ابتليت المجتمعات الريفية التي تنتج معظم المنتجات الزراعية في نيجيريا بالصراعات الطائفية. أحد هذه الصراعات هو الصراع بين طائفتي Share/Tsaragi . حيث تم تقييم تصورات المزارعين حول تأثير النزاعات بين الطوائف على الإنتاج الزراعي في مجتمعات Share و Tsaragi في مناطق الحكم المحلي Edu و Ifelodun في Kwara . أخذت العينات بطريقة عشوائية من ثلاث مراحل لاختيار ١٢٠ شخصا للدراسة. تم جمع البيانات من خلال مقابلتهم . تم استخدام مقياس ليكرت من خمس نقاط لترتيب تصورات المزارعين مع خمسة (٥) كأعلى استجابة و (١) كأقل استجابة. أوضحت النتائج أن ٦٨٪ من المجيبين هم فوق ٤٠ سنة وأن ٧٠٪ يقيمون في المجتمعات المحلية لأكثر من ١٠ سنوات. أدرك المجيبون أن تضارب الموارد الذي يحد من قضية الحدود ، والمطالبة بملكية الأراضي الخصبة في منطقة Fejewe ، وتحويل الأراضي المستأجرة إلى ممتلكات شخصية من بين أمور أخرى هي أسباب النزاعات داخل المجتمعين. وكشفت النتائج بالتساوي تصورات المجيبين حول آثار النزاعات في المنطقة لتشمل ندرة الأراضي الصالحة للزراعة ، وتشريد المزارعين ، وتدمير الأراضي والمحاصيل ، وندرة الغذاء وتدمير المنتجات المخزنة وغيرها. أظهرت الدراسة العلاقة بين الخصائص الاجتماعية والاقتصادية المختارة والتصورات .وتخلص هذه الدراسة إلى أنه يجب أن يكون هناك ترسيم واضح للحدود داخل الطائفتين ، ورفع الحظر المفروض على بعض الأراضي الصالحة للزراعة التي استولت عليها الحكومة وتعزيز المهن الثانوية الأخرى لتخفيف الضغط على الأرض.

لكلمات المفتاحية: الصراع، المزارعون، تصور، ولاية كوارا، Share, Tsaragi

Introduction

The study of peace and conflict has become a global imperative due to the mere fact that the two relate to the condition that defines and shape human existence and wellbeing in the present world (Best, 2004). According to Donohue et al. (1992), conflict is a situation in which people in social interaction express differences in the process of achieving their goals and need. It is inherent in all kinds of social, economic and political settings that are characterized by ethnic, religious, cultural and other forms of pluralism.

The west Africa sub-region, particularly Nigeria, has experienced significant upsurge of inter-communal conflicts which are characterized by: (i) the inter-dependence and inter-communal relations between two or more communities, (ii) the proximities of each community to the other bringing them to share a common boundary, (iii) the presence of scarce resources that each of the communities have claim of ownership and (iv) the prioritization of win-lose perspective in which the goal of each party is to secure a winner takes all advantage (Olabode and Ajibade, 2004). Oboh and Hyande (2006) described the communal conflict as involving two or

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Table 1. Socio-economic characteristic of respondents

Characteristic	Frequency	Percentages
Age		
24-39	38	31.7
40-55	42	35.0
56-71	34	28.3
>72	6	5.0
Gender		
Male	84	70.0
Female	36	30.0
Year of residence in the Area		
< 3	13	18.8
4-9	23	19.2
10-15	25	20.8
16-21	38	31.7
>21	21	17.5
Educational Attainment		
No formal education	18	15.0
Adult education	12	10.0
Primary education	31	25.8
Secondary education	35	29.2
Tertiary education	24	20.0
Farming system		
Crop farming	93	77.5
Animal husbandry	11	9.2
Mixed farming	16	13.3
Religion		
Christianity	37	30.8
Islam	78	65.0
Others	5	4.2

Source: field survey, 2018

more communities engaging themselves in disagreement or act of violence over issues such as claims for land ownership, religious and political differences leading to loss of lives and destruction of properties. Communal violence (sometimes inter-communal violence) is a situation where violence is perpetrated across ethnic lines, and victims are chosen based upon ethnic group membership (Horowitz, 2000).

Inter-communal conflicts have plagued rural communities which produce the bulk of agricultural produce in Nigeria. One of such conflicts is Share/Tsaragi inter-communal conflicts. The inter-communal existence of Yoruba of Share and Nupe of Tsaragi in Kwara State, Nigeria, was dated back when rampaging war brought them together at their present location (Alao, 2012; Gazette of Ilorin, 1921). The communities had been living together peacefully until when resources were becoming scarce to satisfy the need of the two communities and when the Ifelodun Local Government Area (LGA) was created in 1976. The two communities started to identify their cultural differences and began exercising authority and control over farmland and water resources. More so, the boundary between the two communities was not well defined during the reorganization exercise which ceded nine villages to Tsaragi (Memorandum submitted to Tsaragi, Ref: TSEADA/TSG/02/vol1/5, 2010). The earlier friction occurred in 1948 among women as a result of the denial of access to water sources and claiming of ownership by the two communities. Also, when Ifelodun LGA was created in 1976, owing to the preponderance of Yoruba in the LGA, the Nupes (Tsaragi) started agitating to be merged to Nupe dominated LGA.

In the year 2000, these resilient communities clashed over farmland in the surrounding area of Fejewe, which claimed many lives and property worth millions of naira. Again in 2008, another violent clash occurred over ethnic identity and non-implementation of the recommendation of inquiry committee (Alao, 2012; Abdulwahab, 2008). In December 2015, another violent clash occurred between the two communities which claimed many lives and many properties destroyed. This news was reported by major Newspapers in Nigeria (see the Nigeria Vanguard 20/12/2015 and January 10 2016; The Nation 19 & 20/12/2015 and 2nd February 2016). The impacts of all these clashes were the destruction of lives and properties worth millions of naira, displacement of indigenes of both communities and destruction of farmland and farm produce.

Many research works have been carried out on inter-communal conflicts in Nigeria (Akinteye et al., 1999; Albert, 1999; Best et al., 1999; Olabode & Ajibade, 2004; Alao et al., 2012). These research works laid emphasis on historical antecedent to the conflict, its progression into crisis and possibilities of conflict resolution. However, not much focus was given to the influence of the conflict on agricultural production. The new dimension on-farm-related conflicts has been recognised to be a threat to national peace and stability as well as food security in Nigeria. Therefore, there is an urgent need for the identification and analysis of the influence of these inter-communal conflicts on agricultural production in the study area. As a result of the preceding, this study was designed to assess perceptions of farmers on the influence of inter-communal conflicts on agricultural production in Share and Tsaragi communities of Kwara State, Nigeria. The specific objectives were to describe the socio-economic characteristics of the farmers in the study area, identify the causes of inter-communal conflicts in the study area, and examine the effects of these conflicts on food production in the study area. In this

Table 2. Perceived causes of Share/Tsaragi Inter-communal conflicts

Variables	Mean	SD	Rank
Perceptions of farmers agreeing			
Boundary issue	4.70	1.68	1
Claim of ownership of fertile farmland at Fejewe	4.32	1.61	2
Conversion of leased land to personal property	4.19	1.58	3
Competition for land for arable crop production	4.16	1.56	4
Poverty and Unemployment	4.14	1.57	5
Incompatible values	3.58	1.46	6
Perceptions of farmers undecided			
Poor governance	3.23	1.39	7
Support from external interest	3.08	1.36	8
Lack of equal opportunity	2.78	1.29	9
Poor social interaction	2.78	1.25	10
Government involvement	2.68	1.26	11
Competition on land for grazing	2.57	1.24	12
Perceptions of farmers disagreeing			
Language barrier	2.45	1.21	13
Non-implementation of committee of inquiry recommendation	2.32	1.18	14

Source: field survey, 2018

study, it was hypothesized that: (i) There is no significant relationship between the frequency of inter-communal conflicts and socio-economic characteristics of the farmers in the study area, and (ii) There is no significant relationship between the inter-communal conflicts and availability of land for agricultural production.

Materials and Methods

Study Area

This study was carried out in Kwara State, Nigeria. The State has a land area of about 32,500 km square, and a population of about 2,371,089 based on the National Population Census (2006). It is situated between latitude 7°45'N and 9° 30'N and longitude 2° 30'E and 6° 25' of the equator. The state has sixteen Local Government Areas (LGAs) with administrative capital in Ilorin. Share and Tsaragi are located in Ifelodun and Edu local government Areas of Kwara State respectively. The two communities are located on latitude 4° and 8° N and longitude 8° and 9° east of the equator.

The area is characterized by wet and dry seasons with rainfall ranging between 1000 mm and 1500 mm, and average temperature varies between 30°C and 35°C, while relative humidity ranged from 35% to 60%. The primary source of livelihood of both communities is farming with emphasis on cultivation of crops such as rice (Oryza sativa), cassava (Manihot esculenta), yam (Dioscorea alata), maize (Zea mays), guinea corn (Sorghum vulgare cv. durra), groundnut (Arachis hypogaea), cowpea (Vigna unguiculata) okra (Abelmoschus esculentus) and various kind of leafy vegetables (KWADP, 2015).

Sampling Technique and Sample Size

The population of this study consists of all farmers in Share and Tsaragi communities of Kwara state. Three-stage random sampling procedures were used to obtain one hundred and twenty (120), respondents. In the first stage, two Local Government Areas that experienced conflicts were purposively selected. In the second stage, one community that is involved in the inter-communal clash was selected from each LGA. Then sixty (60) farmers were purposively selected from each community to give a total of one hundred and twenty (120) respondents. Data were collected with the aid of a well-structured questionnaire using interview schedule technique to elicit information from the respondents on socio-economic characteristics such as age, marital status, years of residence in the community, educational level, perceived causes of the conflicts and perceived effect of the conflicts on agricultural production. Perceived causes and effects were measured on a five-point likert-type response scale of Strongly Agreed (SA), Agree (A) Disagree (D), Undecided (UD), Disagree (D), and Strongly Disagree (SD).

Table 3. Perceived effects of inter-communal conflicts on agricultural production

Variables	Mean	SD	Rank
Perceptions of farmers agreeing			
Scarcity of arable land	4.32	1.60	1
Displacement of farmers	3.97	1.54	2
Destruction of farm crops	3.71	1.49	3
Scarcity of food	3.70	1.48	4
Destruction of stored produce	3.66	1.47	5
Perceptions of farmers undecided			
Problems of transporting farm produce	3.38	1.42	6
Scarcity of hired labour	3.08	1.36	7
Problems of accessing farm inputs	3.07	1.35	8
Decline in animal annual yield	2.93	1.32	9

Source: field survey, 2018

Data Analysis

Data collected were analyzed using descriptive analysis such as percentage, frequency counts, while Pearson correlation analytical tool was used to test the hypotheses set up for the study. Farmers' perception on the influence of conflict on agricultural production in the study area were evaluated by asking the respondents to indicate the extent of their agreement with each indicator using 5-point Likert-type continuum of the scale of SA, AG, UD, D, SD with a weight loading of 5, 4, 3, 2 and 1 for the statements. For each variable a weighted mean was obtained as follows:

$$WM=\{(fSA*5) + (fAG*4) + (fUD*3) + (fD*2) + (fSD*1)\}/n$$

where WM is weighted mean, F is frequency, values 5, 4, 3, 2, 1 is attached weights, n is total number of statements. This study adopted Joshua et al. (2014) perception analysis, the means of all indicators were categorized as follows: 4.50-5.00 = SA, 3.50- 4.49 = AG, 2.50-3.49 = UD, 1.50-2.40 = D, 1.00-1.49 = SD

Results and Discussion

Socio-economic characteristic of respondents

The socio-economic characteristic of respondents (Table 1) shows that 66.7% is between 24-55 years old while the average age was 46.6 years. This indicated that they were at the active economic age and that the respondents were old enough to understand the conflict between share and Tsaragi concerning the causes, implication and effects on agricultural production within the two communities. The majority of the respondents (i.e. 70%) were male. This suggests that the male was predominant farmers in the study area. The result equally shows that the majority (i.e. 70%) of the respondents have been residing in the area for more than ten (10) years. In terms of educational attainment, 84% of respondents have one form of education ranging from adult to tertiary education. The higher level of education has been known to widen one's exposure. Therefore, it may be affirmed that the majority of the respondents had adequate exposure to the inter-communal conflicts between the two communities. They are equally small-scale farmers as 53% of them had farm size between 1-10 acres as 76% engaged in crop farming. This indicates that crop farming is the predominant occupation of the two communities.

Perceived Causes of Share/Tsaragi Inter-communal Conflicts

Fourteen indicators/variables (as shown in Table 2) on the various causes of conflicts between share and Tsaragi were evaluated after farmers' responses. Their perceptions were calculated by obtaining a weighted mean of the response and later ranked accordingly. The overall result showed the number of variables agreed upon by the respondents as the causes of conflicts was 43%, disagreed on 43% and undecided on 14% (Figure 1). Findings shows that respondents perceived the conflict between the two communities to be over resource use which bordered on boundary issue which was ranked highest, followed by claim of ownership of fertile farmland at Fejewe, conversion of leased land to personal property (contested ownership), competition for arable land for crop production, poverty and unemployment, and incompatible values in that order (see Table 2). This indicates that land which is a major factor of production in agriculture especially crop production was at the center of the conflicts between the two communities. This confirms the earlier results by Ani et al. (2015) and which showed that conflicts were caused as a result of boundary issues and contested ownership.

However, respondents were undecided on six vari-

Table 4. Correlation between respondents socio-economic characteristic and inter-communal conflict.

Variable	r-value	p-value
Educational level	-0.847**	0.000
Secondary occupation	-0.871**	0.004
Year of residence in the area	-0.274**	0.006
Ave. Monthly income	0.131	0.153

(**) Significant at 5% level

ables (see Table 2), which include poor governance, support from outside interest, lack of equal opportunity, weak social interaction, government non-interference and competition on land for grazing. These were ranked 7th-12th in the table. This indicates that respondents perceived that there is no strong reason to link these variables and conflicts in the study area. According to them, no external influence/ interest on their land and people had intermarried between the two communities which made them interact freely when there were no issues. Only two of the variables were out-rightly disagreed with by the respondents (i.e. 14%). These are the language barrier and non-implementation of committee recommendations. This shows that language was not a problem among the people of the study area as the majority of them can speak Yoruba even among the Nupes. Moreover, the respondents may not be aware of what recommendations were submitted to the government for implementation.

Table 5. Perceived effect of Inter-communal conflict on non-availability of farmland

Variable	r-value	p-value
Perceived effect of inter-comm. Conflict		
Availability of arable farmland	0.983**	0.005

(**) Significant at 5% level

Perceived effects of Inter-communal Conflicts on Agricultural Production

Nine indicators/variables were listed on effects of the conflicts on agricultural production in the study area (Table 3). The perception of respondents was calculated as above and then ranked. The result shows that five variables were agreed upon as the effects of the conflicts representing 56% while 44% was undecided. The result reveals (Table 3) that respondents perceived scarcity of land as the most felt effect of the conflicts between the two communities partly because of an outright ban of cultivation on some of the disputable arable lands in the area by the government. Farmers were displaced during and after every clash in the area; farms and crops were destroyed which later led to the scarcity of food even as stored produce was not left out during the conflicts. This indicates that the conflicts have very adverse effects on human development and should be handled carefully to avert unnecessary famine and food insecurity in the state and particularly in the study area. Respondents were, however, undecided on problems of transporting farm produce, scarcity of hired labor, problems of accessing farm inputs, and decline in annual animal yield. This im-

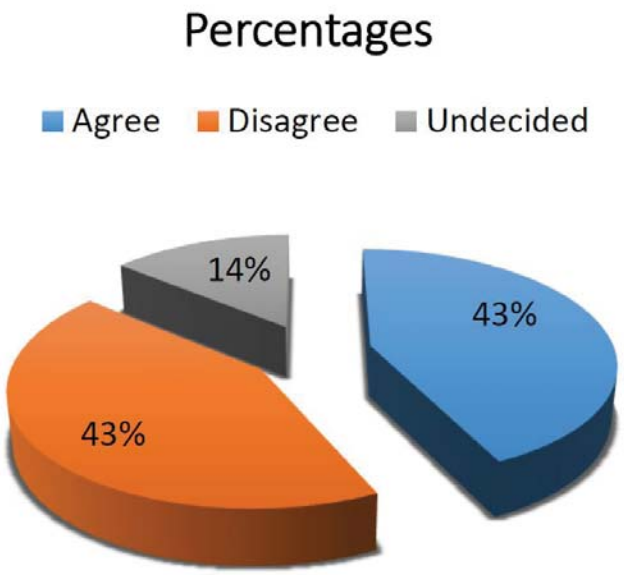

Figure 1. Categorized perceptions on the causes of conflicts between Share and Tsaragi communities

plies that though these are problems respondents could not link them to the effects of the conflicts.

Test of Hypotheses

Pearson Product Multiple Correlation was used to test the hypotheses set up for the study. The result in Table 4 shows that there was a significant but inverse relationship between educational level, secondary occupation and conflicts. It can, therefore, be deduced that farmer's education could contribute to the reduction of inter-communal conflicts in the area as this will give them exposure on how to manage conflict and diversify their source of income. This is in agreement with the observation of McCafferey (2005), who noted that peace and conflict resolution at the community level could be facilitated through increased education.

Similarly, secondary occupations with negative loading imply that if respondents were encouraged to take up other occupations in the area, there might be a gradual reduction of the conflicts between the two communities as this will reduce pressure on land and get them to engage in other non- farm activities. Year of residence equally shows a significant but inverse relationship which can be inferred that the more the respondents stayed in the area, the more they will be able to understand the dynamics of the conflicts and able to resolve or evade its consequences/effects.

Conversely, the second correlation (Table 5) shows a positive relationship between perceived effects and no availability of arable farmland. This implies that the conversion of arable farmland to the buffer zone which makes the farmland inaccessible to farmers, contributes to the frequency of conflicts in the study area. This is consistent with the findings of Dohrn (2008) as insecure land tenure impedes fair resource management which could lead to conflict.

Conclusion and Recommendations

This study has shown that the conflicts in the two local governments were as a result of inadequate farmland for agricultural purposes, which may substantially affect food security if not urgently handled. Furthermore, sustainable development goals (SDG) one, two and three which aimed at ending hunger, poverty and ensuring healthy lives and promote well-being for all at all ages might be a mirage. Below are the recommendations of the study: (i) The government should, as a matter of urgency, lift the ban on some of the arable farmlands seized from the farmers and share equitably between the two communities, (ii) Set up a peace committee among the two communities to resolve any issue before it gets out a hand, (iii) The head of each community or his representative, should be part of the committee, (iv) members of the communities should be encouraged to engage in off-farm activities to lessen competition for agricultural land and thereby reduce the deleterious effects of conflict, (v) Proper boundary delineation should be carried out to douse future tension.

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RESEARCH PAPER

Dynamics of Physical Capital in Artisanal Fisheries and Policy Implications

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ديناميات رأس المال المادي في مصايد الأسماك الحرفية والآثار المترتبة على السياسات

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ABSTRACT. The dynamics of physical capital stock and net investment in artisanal fisheries of Al-Seeb, a coastal fishing town of Muscat Governorate, were examined covering the period 2004-2013. Data was gathered from two sources namely the Ministry of Agriculture and Fisheries and field survey. A hedonic regression model was used to examine the influence of boat characteristics on the boat acquisition prices and then to derive the growth rate of physical capital stock. Boat characteristics comprising of 'age', 'horsepower (hp)' and 'length (vl)' were found to be statistically significant at the 5% level and carry sign consistent with the economic theory of depreciation and cost respectively. While inter-annual fluctuation of net investment was observed, a positive trend in cumulative investment with an average growth rate of 7.63% was experienced in the fishery during 2004-2013. A crude assessment of the operating costs and the gross revenue of surveyed boat-owners in 2015 showed that on average monthly economic profit of 571±169 SD OMR was received by individual boat-owner, which complements the positive trend in cumulative investment. These findings have important management and policy implications in relation to the effective management of harvesting capacity as well as attract investment in the fishery. Finally, some limitations of the study are discussed along with the indication of potential future research.

KEYWORDS: Sultanate of Oman, Al-Seeb, Capital Stock, Investment, Hedonic Model, Fishing Fleet.

المستخلص:تم في هذه الدراسة فحص ديناميات مخزون رأس المال المادي وصافي الاستثمار في المصايد الحرفية في ولاية السيب بمحافظة مسقط في الفترة ٢٠٠٤-٢٠١٣ وذلك بإستخدام بيانات جمعت من وزارة الزراعة والثروة السمكية وعن طريق المسح الميداني، وقد أستخدم نموذج إنحدار المنفعة لفحص تأثير خصائص القارب على أسعار اقتنائه ثم لإشتقاق معدل نمو رصيد رأس المال المادي. وقد وجد أن خصائص القارب التي تتكون من العمر والقوة والطول كانت ذات دلالة إحصائية عند مستوى ٥٪ وتحمل علامة تتوافق مع النظرية الاقتصادية للإهلاك والتكلفة على التوالي، وبينما وجد تذبذب سنوي في صافي الاستثمار لوحظ وجود اتجاه إيجابي في الاستثمار التراكمي بمتوسط معدل نمو ٧,٦٣٪ في مصايد الأسماك خلال الفترة ٢٠٠٤-٢٠١٣، وقد أظهر تقييم تكاليف التشغيل والإيرادات الإجمالية لمالكي القوارب الذين شملهم الاستطلاع في عام ٢٠١٥ أن مالك القارب الفردي حصل في المتوسط على أرباح اقتصادية شهرية تبلغ ٥٧١ ± ١٦٩ ريال عُماني، وهو ما يؤكد الاتجاه الإيجابي في الاستثمار التراكمي، ولهذه النتائج تداعيات إدارية وسياسية مهمة فيما يتعلق بالإدارة الفعالة لقدرة الحصاد وجذب الاستثمار في المصايد، وفي نهاية الورقة تمت مناقشة بعض قيود الدراسة مع الإشارة إلى البحوث المستقبلية المحتملة.

الكلمات المفتاحية: سلطنة عمان، السيب، رصيد رأس المال، الإستثمار، نموذج المنفعة، أسطول الصيد

Introduction

The twin problems of over-investment and excess harvesting capacity are of major concerns for fisheries in both developed (Zhang et al., 2018; Kirkley et al., 2002; Kirkley and Squires, 2003) and developing countries (Pomeroy, 2012; Purcell and Pomeroy, 2015). These problems are essentially linked to both physical and natural capital (Nøstbakken et al., 2011). The role of physical capital as a produced means of production is well established in economics in general (Hulten, 1991), and in natural resource industries like fisheries in particular (Clark et al., 1979). Frequent occurrence of conflicts in fisheries due to excess capacity (Ahmed et al., 2006), threats to long term sustainability due to capital stuffing (Kirkley and Squires, 1998), and poor economic returns associated with over-investment are some of the legitimate justifications for the need to address the concerns of over-investment and excess capacity in small- and/or large-scale fisheries. This calls for effective management of overcapitalization in fisheries which is crucial for ensuring sustainability in fisheries and the flow of benefits to the society in the long run (Kirkley and Squires, 1998). Furthermore, recognizing the role of small-scale fisheries in development (de Melo Alves Damasio et al., 2016), the financing of small-scale fisheries whether by public or private funds has received attention of policy-makers in recent years both locally (Diffey et al., 2009; Setlur and Arbuckle, 2015) and globally (Heck et al., 2007; Holmes et al., 2014). However, a lack of knowledge about the status of physical capital engaged in fishing will not only undermine effectiveness of management but also introduce uncertainty to the future investment planning for the sector. Hulten (1991) emphasized that an estimate of capital, however imperfect, is crucial to understand the

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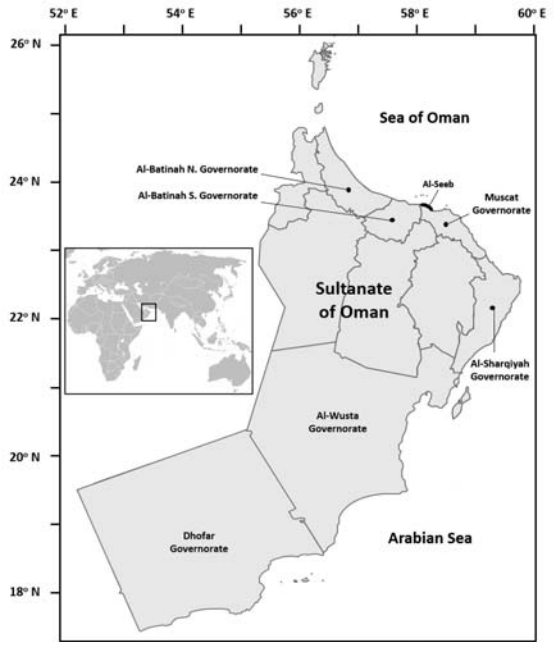

Figure 1. Map of Oman and study location

process of economic growth.

In economics, the description of the term capital has been conceived as deferred consumption, as a stock of durable goods, or as a flow of factor services (Hulten, 1991). In case of fisheries as argued by Squires (1988) that the quantity of capital measured as the flow of services from the number of boats (i.e. physical capital stock) operating in a fishery. The economic theory of capital in fisheries, differentiates three types of capital namely physical (e.g. boat, equipment), natural (fish stock), and human (crews, skills and expertise). The interdependencies of natural and physical capital (Boyce, 1995; Burt and Cummings, 1970) under various conditions such as functional form of investment costs (Boyce, 1995) and degree of malleability (Clark et al., 1979) were studied in fisheries. It was argued that favorable condition of growth in natural capital and other form of economic incentives (e.g. subsidy) leads to investment decisions by fishers and entry and exit behavior of fishers, other things being equal (Le Floc'h et al., 2011). However, it is important to note that exit from fisheries may be difficult due to lack of alternative employment opportunities (Bose et al., 2013; Ikiara and Odink, 1999). Eisenack et al. (2006) argued that for a developing fishery, a time gap existed between the growth of physical and natural capital, and due to partial substitutability between these two forms of capital the growth in physical capital continues even after decline of the natural capital. In general, while the conservation and management of natural capital are the primary objectives of management authorities, the regulatory environment is likely to exert influence on fishers' investment decisions relating to physical capital (Nøstbakken et al., 2011). The capital market imperfections in relation to the borrowing and lending constraint which has particular relevance to small-scale fisheries in Oman where collateral is needed by the financial institutions (Diffey et al., 2009). Other factors affecting investment decision include boat age and size, future revenues, operating costs (e.g. fuel, and labour), stock status of the main target species, and the impact of management measures (e.g. mesh size restrictions) and fleet size (Tidd et al., 2011).

The main objective of this case study were three-fold considering the above-mentioned strategic importance of small-scale fisheries sector in Oman. First, the extent of influence of basic boat characteristics on the acquisition price of boat. Second, an empirical assessment of the dynamics of physical capital stock and net investment in artisanal fisheries during 2004-2013. Third, ascribing importance to the view of 'fishing costs' as a key driver of fishers' investment decisions, among others, and the scarcity of such cost information (Lam et al.,

Table 1. Descriptive statistics of sample used for hedonic regression

Year	Boat	Boat Length (ft)			Boat Price (OMR)			Engine (hp)			Engine Price (OMR)		
	No.	Mean	SD	CV (%)	Mean	SD	CV (%)	Mean	SD	CV (%)	Mean	SD	CV (%)
2004	9	22.89	5.49	23.98	1614.53	971.31	60.16	102.78	92.4	89.90	1938.98	1383.32	71.34
2005	8	23.37	5.66	24.22	1537.87	839.75	54.60	109.37	96.49	88.22	2058.91	1382.51	67.15
2006	8	21.62	4.24	19.61	1361.36	704.28	51.73	88.12	86.89	98.60	2164.27	1842.41	85.13
2007	8	21.62	4.24	19.61	1474.83	803.87	54.51	88.12	86.89	98.60	1697.81	1056.14	62.21
2008	6	21.17	4.21	19.89	1429.34	772.88	54.07	70.83	76.77	108.39	1517.64	1101.61	72.59
2009	6	21.17	4.21	19.89	1676.81	784.26	46.77	70.83	76.77	108.39	1506.67	934.74	62.04
2010	4	22.75	4.57	20.09	1825.53	517.31	28.34	93.75	90.12	96.13	2194.17	1308.22	59.62
2011	4	24.50	4.51	18.41	2142.88	954.55	44.55	143.75	98.1	68.24	2882.25	1189.67	41.28
2012	2	27.00	2.83	10.48	NA	NA	NA	140	84.85	60.61	4053.75	1773.07	43.74

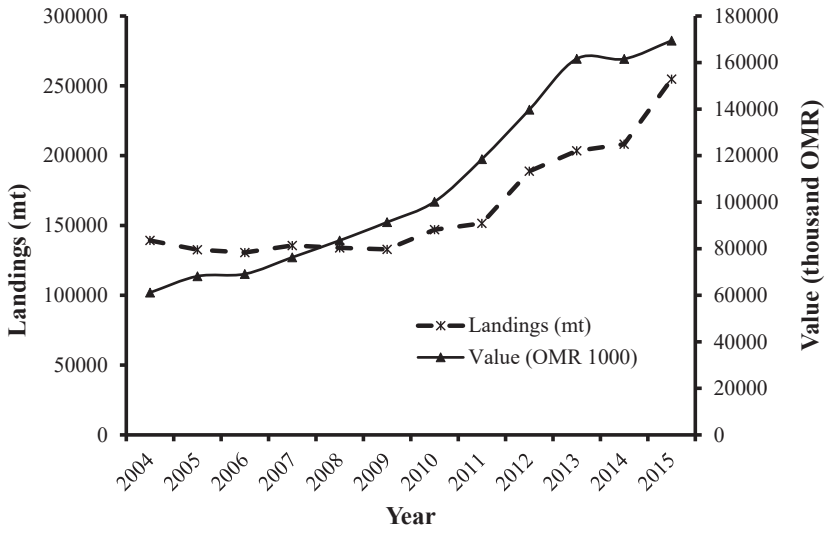

Figure 2. Landings and Value of Artisanal Fisheries, Oman

2011). This study aimed to provide a crude estimate of monthly average operating costs and revenue per boat experienced in 2015. Due to time and resource limitations, the cost estimate was produced using the logbook information obtained from ten owner-operators participated in the study.

From an academic perspective, this study can provide a wider relevance to artisanal fisheries sector in developing countries as this type of quantitative assessment is relatively limited in fisheries literature. In addition, the authors are unaware of any empirical work available or devoted explicitly to address the matter at hand in Oman. More importantly, the lack of knowledge about the existing fleet capacity and the extent of their utilization has been highlighted as major issues, among others, confronting the management of fisheries sector in Oman (Setlur and Arbuckle, 2015; Al-Siyabi and Bose, 2018). In the absence of such information fisheries managers may have the tendency to use the number of operating boats as a measure of capital stock and investment in the traditional fisheries sector. However, such measure would not be adequate as it ignores changes in the quality of such capital over time (Kirkley and Squires, 1998; Kendrick, 1961). So far, there is negligible attempt to collect and compile statistics on either the capital stock or the level of investment in the sector. This study also contributes to the practical realm, as it provides policy makers and investors with knowledge that has the potential to assist in designing effective strategic actions with regard to investment in fisheries. Therefore, this case study not only fills the existing knowledge gap but also complements the existing global literature by adding country-specific information.

The link between aggregate physical capital stock and the harvesting function is well established in fisheries literature, and the dynamic behaviour of physical capital stock is used for explaining the rate of investment associated with fisheries resource use. Therefore, research on capital-stuffing and investment behavior of fishers is valuable as it may provide necessary signal for investment incentives (or dis-incentives) to the private sector which is one of strategic objectives of the sector. The existence of capital market imperfections in relation to the borrowing and lending constraints (Diffey et al., 2009) further rationalizes the importance of the present research to identify market constraints and thereby, enhance the knowledge of the relevant actors to formulate appropriate marketing strategies to accommodate the market dynamics of foreign origin.

Case Study Profile

Fisheries are an integral part of Oman's economy and have occupied an important place in the national policy agenda as the 'Oman Vision 2020' - a long-term development plan, reflects the country's desire to achieve food security, enhance fishers' income, and maximize socio-economic benefits from the sector (Bose et al., 2010; MNE, 2007). The superior economic performance of the sector is a prerequisite for the achievement of these strategic objectives.

Small-scale artisanal fisheries in Oman have been the dominant both in terms of landings and value. During the past decade or so, the sector's contribution to Gross Domestic Product (GDP) has remained stable around 0.6% (at 2000 constant prices) (Bose et al., 2010; Al-Subhi et al., 2013). With the advent of economic diversification policy guided by 'the Vision for Oman's Economy-2020' the fisheries sector has attracted renewed

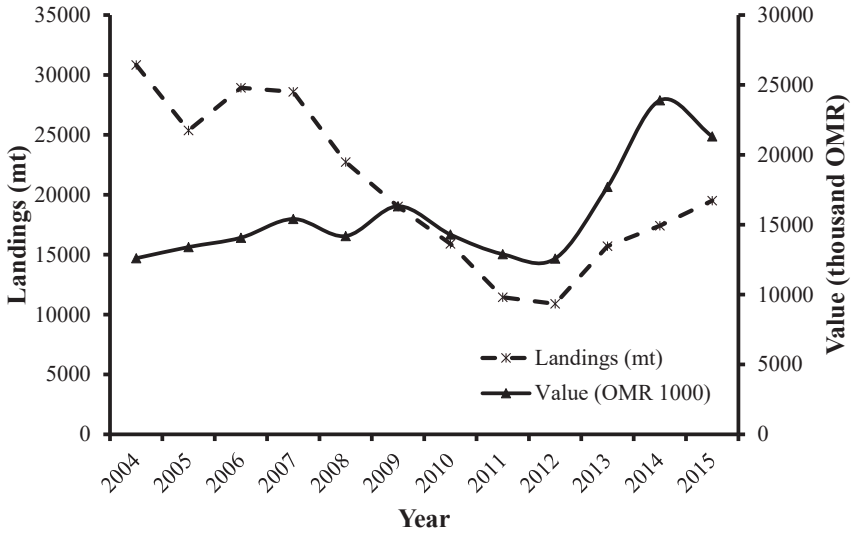

Figure 3. Landings and Value of Artisanal Fisheries, Muscat Governorate

interest and accordingly the authority has been promoting public-private partnerships to attract private investment in the sector. Recently, efforts have been directed to, amongst others, (i) modernize fishing operations and improve fleet performance, (ii) initiate effective fisheries governance mechanisms; (iii) ensure sustainable use of fisheries resources; and (iv) optimize socio-economic benefits from the sector.

The present study conducted in Al-Seeb area which is a coastal fishing town located in the northwest part of the Muscat Governorate. The selection of the study site is influenced by several factors, such as, time and costs, information availability and geographical proximity. It is envisaged that the replication of this study would be useful for generating insights into the pattern of fishery investment and subsequently the design of effective management policies in relation to the future investment in the fishery. Muscat Governorate is one of the seven coastal governorates of Oman (See Figure 1). Muscat Governorate has six Wilayah (Province) namely Muscat (capital city), Qurayyat and Al-Seeb, Bawshar, Al-Amrat and Mutrah.

Figures 2, 3, and 4 exhibit the performance of total fish landings and gross value at the national (Oman), governorate (Muscat) and Wilayah (Al-Seeb) level respectively covering the period 2004-2015. From these figures, it can be noted that at the national level showed a distinct positive trend during the study period, while the same pattern was not evident at the governorate and Wilayah level. However, a resemblance was observed in the behavioral pattern of landings and gross value at the governorate and Wilayah level. Further comparative analysis comprising landings, gross value, fishing boats, and number of fishers are provided in the results section.

Methodology

Data

Ideally, the economic value of capital services should be reflected in rental price. In the absence of such rental market the imputed price of capital services consists of two cost elements, namely the opportunity cost and the depreciation cost associated with the investment and the capital equipment respectively (Jorgenson, 1974; cited by Squires, 1988). There is negligible published database on actual transaction prices of new boats and, therefore, the factory quotation prices for new boats available for the period 2004-2013 were used in this analysis. In the absence of price information for other capital items, such as fishing gears, electrical equipment, and consistent price index series for heavy machinery, the nominal prices of tangible assets such as boats and engine along with the corresponding quality attributes age, boat length, and engine horsepower are considered in this study. Data were collected from two sources. First, the price quoted by Omani companies submitted to the Ministry of Agriculture and Fisheries during 2004-2013 for 53 boats of various sizes (ranging from 16ft to 33ft) and engine types were collected. The acquisition prices were not available. Second, data on registered fishing boats operating in Al-Seeb coastal water along with their characteristics, such as size, engine power, and boat age were collected from the local Ministry office. It was noted that the quoted prices for the same boat size and engine power from different companies differ in a given period due to different technical specification of boats and add-ons (such as warranty condition, and insurance period) coming with the deals. The average value was used to represent such cases. As mentioned earlier, a

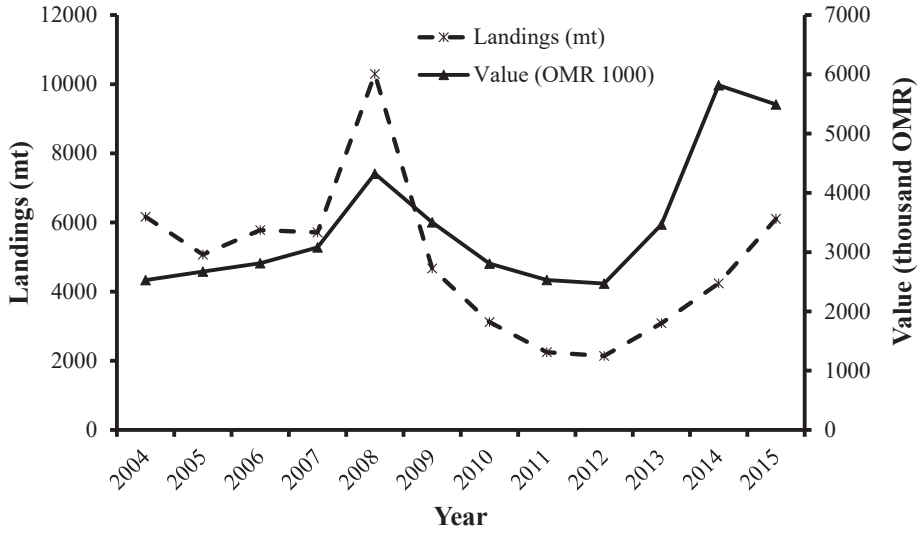

Figure 4. Landings and Value of Artisanal Fisheries, Al-Seeb

simple logbook was prepared (in Arabic) to collect information on basic operation costs. Ten owner-operators nominated by the local office of the Ministry were engaged during February-April 2015 to record costs per trip incurred by individual fisher for two weeks (14 trips per fisher).

In fisheries, boats are treated as a durable but depreciable asset. In estimating physical capital stock and net investment in the New England commercial fisheries (Kirkley and Squires, 1988) followed hedonic regression method and used data on boat characteristics, such as gross registered tonnage (GRT), length, and engine power. The basic idea behind the hedonic approach is to consider a fishing boat as a bundle of characteristics, such as length, age, engine horsepower (hp) and to generate estimate of the missing prices when quality changes.

Empirical Model

The basic model adopted in this study is mainly drawn from the study by Kirkley and Squires (1988). It involves two steps. First, a hedonic model was estimated, and second step growth rate of capital stock was derived by making use of the estimates of implicit price from the first step. Broadly speaking, the hedonic approach hypothesizes that the price of a commodity is influenced by its characteristics (Rosen, 1974). Kirkley and Squires (1988) have argued that the hedonic approach offers an attractive characteristic for estimating capital stock in fishing industries such that it incorporate changes in the quality of capital over time which should be reflected in boat acquisition prices. To represent this, the following Cobb-Douglas functional form was employed.

$$C=\text{constant}.(age)^{\alpha}.(vl)^{\beta}.(hp)^{\gamma}.(\exp)^{u} \quad \text{Eq.(1)}$$

where, C is the list price or acquisition price of boat, age is age of the boat, vl is boat length, hp is engine horsepower, and u is white noise error term. From a theoretical standpoint, the relationship expected between the price and the characteristics are as follows: $\alpha<0$,$\beta>0$,and $\gamma>0$. The hedonic approach is adopted because there is no market information available for used or rented boats. The application of hedonic regression on the available data provides implicit prices of the chosen quality dimensions which would also provide signal to the cost of fishing effort. The age of a boat, was counted from the year the boat was first registered in the boat registry of the Ministry. Size (measured as boat length) and capacity (measured as horsepower) are two important characteristics of fishing boats as they are routinely used for fishing effort measures. The stability of the estimated coefficients obtained from the hedonic regression model would not be tested due to lack of enough observation for each expected period.

In empirical analysis, a linear, semi-logarithmic, and log linear form of the model is frequently used. As there is no a priori reason to assume that price and quality behave in a particular fashion, a Box-Cox transformation was applied to decide whether linear or log-linear functional form of the model fit the data best as follows:

$$x_t^* = \frac{X_t^{\lambda}-1}{\lambda} \quad \text{Eq.(2)}$$

Parameter 'λ' which was determined by the data defines the functional form as: if λ=1, it represents linear speci-

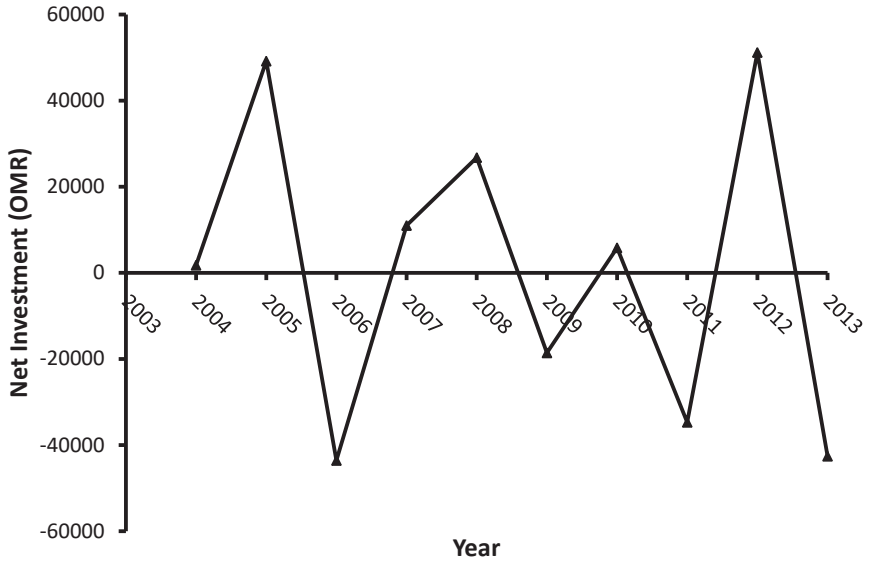

Figure 5. Net Investment in the Fishery during 2004-2013

fication, and if λ=0, it reduces to log-linear specification. Each observation of the dependent variable (i.e. boat price) was first divided by the geometric mean (GM = 3118.32) of the dependent variable and two versions (with and without taking log of the dependent variable) of the regression model (Equation 1) was run. The values of residual sum squares (RSS) were used to conduct a Chi-square test to decide between the linear and log-linear functional forms. The test result (see the result section) supported a log-linear version of the model. As the model is in log-linear form, the resulting regression coefficients can be interpreted as partial price elasticity with respect to the variable concerned, other things being equal. Following Grillches (1961), a semi-logarithmic form of the model (Equation 1) with respect to the age variable was also experimented to accommodate the possibility of geometric patterns of depreciation of boat value with age.

The first data set was used to estimate the parameters from the two versions of Equation 1. At the second-stage, the parameter estimates from Equation 1 were used to generate estimate of capital stock per boat per year based on the second data set that include physical characteristics of the registered boats that have been operating in the Al-Seeb fisheries. The estimated capital stock per boat was then summed for all boats in a year to obtain the estimate of total capital stock. It was assumed that the value of all capital services was the sum of the values of individual capital services (Squires, 1988). Net investment was then calculated as the change in total capital stock per unit of time (i.e. year).

Results

Some relevant comparative statistics are also calculated from the data presented in the Figures 1-3. During 2004-2015, the average growth rate of total landings in Oman was about 5.65% per annum whereas Muscat governorate and Al-Seeb were witnessed negative growth rate of -4.08% and -0.09% respectively. However, during the same period, the positive trends in total value were observed for Oman, Muscat governorate and Al-Seeb, was observed the average growth rate of 9.71%, 4.90% and 7.31%, respectively. Furthermore, for Muscat and Al-Seeb, a downward trend was observed for total landings around mid 2000-2012 and then a positive trend after 2012. The sharpest decline in landings appeared to take place in 2008. With reference to number of fishers and boats positive trends were observed for Oman, Muscat and Al-Seeb but differed in extent, with the average growth rates of 3.40%, 1.60% and 2.41% for number of fishers and 2.03%, 1.61%, and 2.11%, respectively.

The variation in total landings as measured by the coefficient of variation (CV) for Al-Seeb was the highest (45.47%) followed by Muscat governorate (32.94%) and Oman (24.92%). On the other hand, the variation observed in total value was the highest for Oman (37.18%) followed by Al-Seeb (33.49%) and the governorate (22.91%).

The descriptive statistics of the sample used for hedonic regression model are presented in Table 1. It was mentioned earlier that a Box-Cox transformation was followed to decide between the two conventional versions namely the linear and the log-linear models. The estimated χ^2 test value (38.25, 8 df) supported the log-linear specification of the model. Table 2 provides the empirical results of the hedonic regression mod-

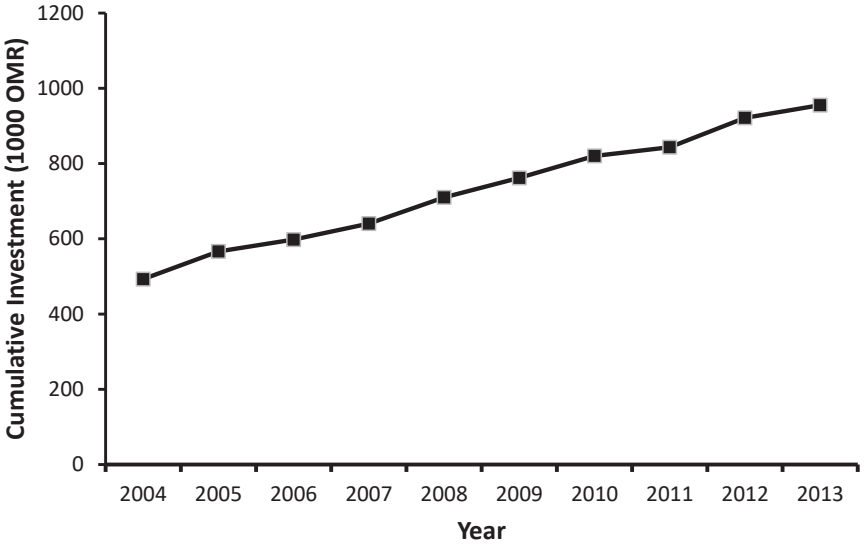

Figure 6. Cumulative Investment in the Fishery: 2004-2013

el specified in Equation 1 in two forms (log linear and semi-log with respect to age) along with the summary statistics and diagnostics. The potential 'non-linearity' in variable was also experimented by including square root term for each variable in to the model but failed to generate any theoretically convincing results.

Some important results can be noted from Table 1. The average boat length was more or less similar across the years, which indicated the degree of homogeneity in small-scale fisheries with respect to boat size. However, the results of boat power exhibited relatively greater degree of heterogeneity in the sample as reflected in the coefficient of variation (CV) estimate. This was because of the fact that some boats were using twin-engine. This was also reflected by the fact that the average price of engine is higher than that of boat except for 2009. Furthermore, the variability (as indicated by CV estimates) of average boat price was lower than engine power and engine price which to some extent consistent with the homogeneity in boat size as mentioned earlier. Apart from higher capital cost (i.e. fixed cost), the use of twin-engine also exerted influence on variable costs due to recent rise in fuel price.

It is noted that a limited number of quality dimensions explain a very large fraction of the total variability of the list price as measured by R^2 value. The coefficient of the variable 'age' carries an expected sign as expected from the economic theory of depreciation and the associated t-value suggests that the variable is statistically significant at the 5% level. The coefficient of age in Model 1 is the elasticity, which is the predicted average proportionate change in boat list price due to proportionate change in boat age, holding other qualities constant. On the other hand, semi-log form the coefficient of age can be interpreted as the percentage change in the boat list price due to a unit change in age, other things being equal.

The variables 'horsepower (hp)'and 'length (vl)' are statistically significant at the 5% level with expected sign that is consistent with the theory of cost which predicted that the boat cost should increase with the increase in the characteristics of power and size. These results are in line with Kirkley and Squires (1998). Furthermore, it is important to note that boats engine power and length have the highest influence on the acquisition price of boats, which is consistent with the descriptive statistics presented in Table 1.

To demonstrate the empirical validity of the model a series of diagnostics has been applied and the results are presented in Table 2. It can be seen that in all cases, the results from the econometric diagnostics do not exhibit any deviations from the classical linear regression properties. The J-B LM test was used to examine the normality of the residuals at the 5% level (Jarque and Bera, 1980). The test statistic follows a χ^2 (with 2 df) distribution and the value of the test statistic indicates that the null hypothesis of normality cannot be rejected. The B-P-G test was used to test the null hypothesis of homoscedasticity of the residuals at the 5% level (Breusch and Pagan, 1979; Godfrey, 1978). The test statistic follows a χ^2 (with 2 df) distribution and the test value was insignificant at the 5% level thereby the null hypothesis of homoscedastic error variances cannot be rejected. The Lagrange Multiplier (LM) test statistic with two lags was used to test the null hypothesis of no autocorrelation at the 5% level. The test result supported the null hypothesis of no autocorrelation. Comparing the output of summary statistics, forecast performance, and model selection criteria, it can be

Table 2. Empirical results of the hedonic regression model

Model 1 (log-log)			Model 2 (Semi-log w.r.t. age)		
Variable	Coefficient	t-value	Variable	Coefficient	t-value
ln(age)	-0.23	-4.61	age	-0.03	-4.50
ln(hp)	0.49	9.97	ln(hp)	0.49	9.85
ln(l)	0.47	2.26	ln(l)	0.49	2.37
Constant	4.98	10.49	Constant	4.71	10.12
Summary Statistics:					
R^2		0.95	0.94		
Residual Sum Square (RSS)		0.78	0.81		
Log likelihood		37.20	36.82		
F statistics (Joint test)		287.50	283.16		
(p = 0.00)			(p = 0.00)		
Regression Diagnostics:					
Normality: LM test($\chi^2$2 df)		2.23 (p = 0.33)	2.27 (p = 0.32)		
Serial correlation: Durbin Watson (D-W)		2.06	2.05		
LM Test ($\chi^2$2 df)		1.52 (p = 0.47)	0.98 (p = 0.38)		
Heteroscedasticity: B-P-G test ($\chi^2$3 df)		0.10 (p = 0.96)	0.11(p = 0.95)		
Forecast Performance:					
RMSE		0.121	0.122		
MAE		0.102	0.103		
MAPE		1.274	1.288		
Model Selection Criteria					
AIC		-1.23	-1.22		
SC		-1.08	-1.07		
H-Q		-1.17	-1.16		

seen that Model 1 performs only marginally better than Model 2. Therefore, it was decided to use the parameter estimates of Model 1 in the second stage.

At the second-stage the parameter estimates from Equation 1 were used to generate estimate of capital stock per boat per year based on the second data set that include physical characteristics of the registered boats that have been operating in Al-Seeb water. The net investment was calculated as the change in total capital stock per unit of time (i.e. year) for the period 2004-2013 and it is presented in Figure 5, which exhibits inter-annual fluctuation in net investment in the fishery. Despite this year-to-year fluctuation, the cumulative of net investment in the fishery experienced a positive trend during 2004-2013 as depicted in Figure 6. Table 3 presents the descriptive statistics of boat characteristics, engine and gear price, maintenance costs, and operating costs per trip per boat gathered from the logbook survey data.

Table 3. Descriptive statistics of boat operation costs, Al-Seeb

Item	Min	Max	Mean	SD
Boat size (feet)	22	25	23.9	1.2
Engine Power (hp)	60	200	103.5	43.5
Engine Price (OMR)	1500	5000	2604.1	1240.6
Fishing Gear (units)	10	83	38.7	25.4
Fishing Gear Price (OMR)	525	5350	2215.9	1471.7
Maintenance Cost (OMR)	150	1250	517.8	332.6
Fishing Trip (hours)	2	19	8.1	3.8
Cost/trip/boat (OMR)				
Fuel	1	67	8.1	9.6
Oil	0.7	23.5	2.4	2.5
Food	0.2	33.5	2.7	3.4
Bait	0.5	15	4.3	3.2
Ice	0.5	8	1.5	1.1
Total	4	75.9	15.4	14.3

Source: Field survey

It is noted that the boats involved in cost analysis ranging in length from 22 feet to 25 feet with an average horsepower of about 104 hp. These figures are within the range presented in Table 1. Furthermore, the largest variability with regard to engine price is consistent with the result presented in Table 1. On average, the fishing trip lasted for about 8 hours. The operating costs include fuel, oil, food bait and ice, and the average cost per trip for individual fisher was estimated to be about 15 OMR. On average, fuel cost has the highest share (about 53%) followed by the bait cost (28%) in the total trip cost. This information is of important use for future research in this area. The highest share of fuel cost has important cost implication for boats that are using twine-engine as stated earlier.

To get an idea about the extent of economic benefits generated from fishing activities, crude estimates of the average monthly net value earned per boat are presented in Table 4 based on the survey data and the data from the statistical yearbook. The gross value is evaluated at farm gate prices of fish species. In the calculation of monthly operational costs per boat, it was assumed that individual boat makes 20 trips per month. Therefore, the estimate was conservative in nature.

Fishers' physical access to market can improve their

Table 4. Crude estimates of the average monthly net value earned per boat in Al-Seeb

Number of boats		521	
Number of fishers		1201	
Cost (OMR)/trip/boat		15.366	
Cost (OMR)/boat/month *		307.320	
Month	Value (OMR)	Value (OMR)/boat	Income (OMR)/boat
January	593000	1138.2	830.9
February	599000	1149.7	842.4
March	448000	859.9	552.6
April	321000	616.1	308.8
May	447000	858.0	550.6
June	370000	710.2	402.9
July	315000	604.6	297.3
August	439000	842.6	535.3
September	467000	896.4	589.0
October	535000	1026.9	719.6
November	500000	959.7	652.4
December	457000	877.2	569.8
Total	5491000	10539.3	6851.5

* assuming that a boat will make 1 trip per day and work for 20 working days a month.
Source: Field survey and MAF data.

profitability, as the prevailing market price was higher than that of farm gate. One of the reasons for the establishment of the central wholesale market in Oman in April 2014 was to give fishers access to market and address pricing inefficiency (Al-Busaidi et al., 2016; Al-Jabri et al., 2015). Furthermore, the extent of profitability was also influenced by the economic value of species caught, which differed from species to species. The monthly net revenue figures ranged from 297 in July (summer month) to 842 in February (winter month). On average, the monthly net revenue per boat was about 571 ± 169 OMR. The findings indicates the fact that fishing has been a profitable profession and this is reflected in the estimates of cumulative net investment. Personal discussion with fishers also revealed the same. However, up-to-date, comprehensive, and quality economic data are required to convey precise assessment of the economic situation of the sector. Expenditure figures presented in Table 4 are only a part of the costs involved in fishing operations. The cost of the boat in the form of depreciation allowance needs to be accounted. In addition, the opportunity costs of capital and the owner's labor and management have to be accounted for in order to arrive at the results of this economic activity.

Discussion

The degree of homogeneity in relation to boat length and the degree of heterogeneity to engine power are consistent with the findings by Al-Siyabi and Bose (2018). At least two important implications of these findings are as follows: (i) the homogeneity aspect may help reduce the conflicts, which arise from unequal catch share between large and small boats, and (ii)) the apparent heterogeneity to engine power along with the highest share of fuel cost (on average 53%) has important economic implications as the use of twin-engine exerts influence on operating costs. In addition, the recent rise in fuel price as noted by Al-Siyabi and Bose (2018) exerted upward pressure on the operation costs.

Higher fuel costs may discourage fishers' future investment decisions in the sector as it could reduce net gain unless it is compensated by the revenue earned through either increase in fish price or increase in total landings, or a combination of both (Tidd et al., 2011). Tidd et al. (2011) found a statistically significant influence of fuel prices on vessel entry and suggested that the vessels would enter at lower costs of subsidized fuel.

The highest degree of variation in total landings measured by the coefficient of variation (CV) and its sharpest decline experienced in 2008 for the study area signal the level of uncertainty in generating revenue from fishing. This is reflected by the variability in fishers' income as shown in Table 3. The authority may promote diversification rather than specialization of fishing portfolio through fishers' participation in additional or more diverse fisheries to mitigate seasonal variation in their income. This approach is common in fisheries. Anderson et al. (2017) proposed such strategy to buffer against income variability due to seasonality in fisheries. In a local context, alternative strategic initiatives such as aquaculture development (Al-Siyabi and Bose, 2018) and fishing tourism (Al-Busaidi et al., 2016) are also suggested to enable fishers to choose related but alternative profession. This type of approach would also help reducing fishing pressure and if economically viable would encourage relatively inefficient fishers to exit from harvesting operations.

The results of the hedonic model in relation to boat age, length and horsepower are consistent with the theoretical predictions and have important management implications. The statistical significance of the variable boat 'age' can be related to operational efficiency of the fleet as older vessels are expected to be less efficient than the newer one. Tidd et al. (2011) argued that older vessels are more likely to exit the fishery. The statistical significance of the variables 'horsepower (hp)' and 'length (vl)' predicted that the boat cost would increase with the increase in the characteristics of power and size. The results are also consistent with other empirical studies (Tidd et al., 2011). The implication of these results is that boat size and horsepower together with the high

fuel costs may affect stock status and the economic viability of fishers, which in turn discourage future investment in fisheries.

With regard to the parameter estimates of the model, one may argue that the use of list prices of boats and engines introduces bias in estimates depending on the extent to which they deviate from the actual acquisition price. Furthermore, prices of used boats or rental market were not available. Due to a lack of sales data (new or used), validity of parameter estimates with specific reference to boat age could not be checked. It should also be noted that the boat acquisition price series were not inflation adjusted due to a lack of suitable (e.g., price index for equipment) and consistent price index for the study period. However, this may not undermine the results. It is argued that available machinery and durable equipment price index failed to incorporate quality changes adequately (Griliches, 1961), and therefore the process of deflation can introduce errors in measurement of price variable (Hulten, 1991). Usher (1980) mentioned the choice of price index reflects the equivalent performance of the old and new capital goods which is contrary to the real situation. The estimated parameters may be upward bias due to the use of quoted price rather than the acquisition price. However, this upward bias may be partly compensated as other components of capital costs, which are not included in the present analysis.

While inter-annual fluctuation in net investment in the fishery is observed, the cumulative investment trend was positive. The negative net investment could be influenced by various factors. For example, disinvestment in 2011 may be due to negative growth in landings as depicted in Figure 4 and social upheaval experienced in the country (Valeri, 2015). On the other hand, the positive net investment was influenced by the influx of new entrant. For instance, 25 new boats added to the registry in 2005. The positive trend in cumulative net investment for the period 2004-2013 reflects the overall dynamics of physical capital. Therefore, a convincing case can be made to support government investments and develop infrastructure. The positive trends in relation to number of fishers and boats were observed for Oman, Muscat and Al-Seeb signal economic potential of fisheries. In a recent case study, Al-Siyabi and Bose (2018) noted the existence of under-utilized fishing capacity and the presence of technical inefficiency in the utilization variable inputs (i.e. crews and fishing time) in small-scale fisheries. Based on these findings, Al-Siyabi and Bose (2018) argued that there was potential for improving production without having to incur any additional physical capital costs. Therefore, this signal of economic potential can be used as a tool to attract private investment in the sector, which is one of the strategic goals of the sector as mentioned earlier. However, a precautionary approach needs to be adopted and any effort to increase investment must strike a balance with the stock status. Otherwise, the call for public-private partnership can inevitably be limited.

While the findings in this study have important strategic implications with regard to future course of actions in the context of investment in small-scale fisheries, there are a number of limitations of this paper that need to be mentioned. First, a variety of potential variables such as fishing gear and equipment, and shore-based tangible assets like cars and buildings, are not considered as the data for such variable are not readily available. Second, the estimate of capital stock is based on the registered fishing boats in the local office, which is different from the number of boats as published in the yearbook. This discrepancy is due to the time lag in license renewal requirements. Third, estimates generated in this study used registered boats of Al-Seeb fisheries only, therefore, it should not be treated as an indication of net investment in the overall fisheries sector of Oman.

Conclusion

This case study uses a hedonic approach for examining the dynamics of physical capital stock and net investment in Al-Seeb artisanal fisheries. In spite of the above-mentioned limitations, the study has identified important information gaps in this area of research, set the groundwork for a more complete research study in the future, and form the basis for generating insights into the pattern of fishery investment and subsequently the design of effective management policies in relation to the future investment in the fishery. While the present case study has provided useful information, the robustness of empirical data and conclusions needs to be verified with further studies to a point where generalization of results can be accomplished. There is a great deal of room for further research particularly in the area of inclusion of physical assets other than boat and engine, inclusion of add-on characteristics in the quoted price, larger samples covering the whole sector, and improved method of inferring depreciation rates of boats. They could provide the authority with essential indicators/estimates which could be used in promoting and making investment decisions. Furthermore, it could motivate the data collection agencies to create a database and provide a better basis for making informed decisions.

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RESEARCH PAPER

Antibacterial Activity and Chemical Composition of Crude Extract and Oil of *Zygophyllum (Fagonia) luntii* (Baker) 1894 (Family Zygophyllaceae)

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النشاط المضاد للبكتيريا والتركيب الكيميائي للمستخلص الخام وزيت زيغوفيللوم (فاجونيا) لونتي (بيكر) ١٨٩٤ (عائلة زيغوفيللاسي)

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Abstract. Wild plants such as *Zygophyllum luntii*, from the Zygophyllaceae family, have traditionally been used for medicinal purposes in Oman. The present study investigated (i) the antibacterial activity of the crude extracts (leaves, stem and roots) and the oil (leaves); and (ii) the hydrocarbon contents and fatty acid methyl ester (FAME) components from *Z. luntii*. These extracts were tested against *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* using well diffusion assays utilizing Muller Hinton Agar (MHA). Antibacterial activity was observed with the *Z. luntii* leaf extract and significant differences (F=14.06, df=2, P=0.002) were found among *E. coli*, *P. aeruginosa* and *S. aureus*. The highest inhibition was observed against *P. aeruginosa*, with an inhibition zone of 15.5 ± 2.6 mm, followed by *E. coli* and *S. aureus* with inhibition zones of 11.3 ± 1.5 mm and 3.5 ± 4.7 mm, respectively. The *Z. luntii* extracts showed effectiveness within 50-60% against *E. coli* and *P. aeruginosa* as compared to Ciprofloxacin. The hydrocarbon contents and the FAME components of the extracts were determined with Gas Chromatography-Mass Spectrometry (GC-MS). A total of 20, 19, and 8 compounds were detected from leaf, stem, and root extracts, respectively. Heneicosane, docosane, and tricosane were found in the highest concentration in the leaves, HOP-22(29)-EN-3.BETA.-OL and β-sitosterol were found in the stems, and docosane and tricosane were found in the roots of *Z. luntii*. Nine types of fatty acids methyl esters were detected in the oil extracted from leaves with methyl esters of palmitic acid, linolenic acid, and oleic acid constituting 90% of the oil. This is the first report on antibacterial activity and chemical composition of *Z. luntii*.

Keywords: *Zygophyllum luntii*, Antibacterial activity, Gas Chromatography- Mass Spectrometry (GC-MS), Fatty Acid Methyl Ester (FAME)

المستخلص: يتم استخدام النباتات البرية مثل زيغوفيللوم لونتي *Zygophyllum luntii* المسماة السقطرية ، من عائلة زيغوفيللاسي Zygophyllaceae بشكل تقليدي في عمان للأغراض الطبية. بحثت هذه الدراسة عن النشاط المضاد للبكتيريا للمستخلصات الخام من الأوراق والساق والجذوروالزيت المستخلص من الأوراق. تم الكشف على مركبات من الهيدروكربونات والأحماض الدهنية في مستخلص الأوراق لنبتة *Z. luntii*. تم اختبار هذه المستخلصات ضد بعض الأنواع من البكتيريا مثل *Escherichia coli* و *Staphylococcus aureus* و *seudomonas aerugi-nosa* بطريقة فحوصات الانتشار الجيد بإستخدام أجار مولر هينتون المناسب لنمو هذه الأنواع من البكتيريا. لوحظ نشاط مضاد للجراثيم من مستخلصات نبات *Z. luntii* ووجدت فروق ذات دلالة إحصائية بين *E. coli* و *P. aeruginosa* و *S. aureus* . فقد لوحظ أعلى تثبيط ضد *P. aeruginosa* مع منطقة تثبيط ١٥,٥ ± ٢,٦ مم ، تليها *E. coli* و *S. aureus* مع مناطق تثبيط ١١,٣ ± ١,٥ مم و ٣,٥ ± ٤,٧ مم على التوالي. أظهرت مستخلصات *Z. luntii* فعالية في حدود ٥٠–٦٠ ٪ ضد *E. coli* و *P. aeruginosa* مقارنة مع النموذج التجاري سيبروفلوكساسين. تم تعريف مركبات الهيدروكربونات والأحماض الدهنية في المستخلصات بإستخدام جهاز الفصل الكروماتوجرافي الغازي المرتبط بمستشعر الطيف الكتلي (GC-MS) . تم الكشف عن مامجموعه ٢٠ و ١٩ و ٨ من المركبات من مستخلصات الأوراق والساق والجذور على التوالي. تم العثور على الهيدركربونات هينيكوسان و دوكوسان وتريكوسان كأعلى تراكيز للمركبات في الأوراق ، وتم العثور على مركب HOP-٢٢ (٢٩) –EN–٣. BETA.–OL ومركب بيتا سيستيرول في السيقان ، كما تم العثور على مركبات دوكوسان وتريكوسان في الجذور الموجودة في *Z. luntii* . تم الكشف عن تسعة أنواع من الأحماض الدهنية في الزيت المستخرج من الأوراق مع نسب عليا لمجموع تراكيز حمض البالمتيك وحمض اللينولينيك وحمض الأوليك شكلت مانسبته ٩٠٪ من الزيت. يعتبر هذا هو التقرير الأول على مستوى الدراسات البحثية عن النشاط المضاد للبكتيريا والتركيب الكيميائي لنبات *Z. luntii*.

لكلمات المفتاحية: زيغوفيللوم لونتي ، النشاط المضاد للبكتيريا ، جهاز الفصل الكروماتوجرافي الغازي المرتبط باالطيف الكتلي (GC-MS) ، الأحماض الدهنية الميثيل إستر (FAME)

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Introduction

Around 80% of the world population use traditional medicine which is often based on medicinal plants (Martins, 2013; Oyebode et al., 2016). Around 75% of commercial drugs launched in the world

global market yearly are extracted or isolated from natural resources and about 25% of the prescribed pharmaceutical drugs are based on plant chemicals (Orhan, 2012). Plants are the major source of secondary metabolites, which are used to treat various diseases (Hossain et al., 2013; Akhtar et al., 2017; Raqiya and Hossain, 2017; Asma et al., 2017; Hossain, 2018; Said et al., 2018). Medicinal plants are found in many places in the world; however, they are found more in tropical regions (Al-Salt, 2012).

Family Zygophylllaceae includes many medicinally important plants spices including several species of *Zygophyllum*. *Zygophyllum* have antitumor, antioxidant and analgesic properties, and have been used for the treatments of cancer, fever, asthma, urinary discharges, toothache, stomach problems and kidney diseases (Ahsan et al., 2007; Satpute et al., 2009). *Zygophyllum* species were found to be potent antifungal and antibacterial agents (Zhang et al., 2008; Gupta et al., 2009) and contained many biologically active chemical constituents, such as alkaloids, saponins, terpenoids, sterols, flavonoids, coumarins and trace elements (Beier, 2005).

Zygophyllum luntii distribution is restricted to the Horn of Africa region, including Djibouti, Oman, Somalia and Yemen (Beier, 2005). It grows on sand as well as gravel, from sea level up to 1950 m altitude. In Oman, *Z. luntii* is found in the foot of Dhofar mountains along with several other species of *Zygophyllum (Z. bruguieri, schweinfurtii, indica, mahrana* and *ovalifolia)* (Mosti, et al., 2012).

The current study explored two objectives. Firstly, antibacterial activity of the *Z. luntii* extract (leaves, stems and roots) and leaf oil against *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* were investigated. Secondly, hydrocarbon contents of the plant extracts and lipids fatty acid methyl ester were determined (FAME) components of the oil extracted from leaves by gas chromatography- mass spectrometer (GC-MS).

Materials and Methods

Collection and Preparation of Plant Materials

Roots, leaves, and stems of *Z. luntii* were gathered from the Botanical Garden at Sultan Qaboos University, Oman. These parts were cleaned with tap water followed by distilled water to remove any dust and soil. The plant parts were then further divided into two portions; one was dried in the oven at 70°C for 8 h and then ground into a fine powder and other portion was kept fresh in a refrigerator at 4°C.

Plant Extracts Preparation for Anti-bacterial Test

The dried powder of the leaves, stem and roots was dissolved in 70% methanol (1:3, w/v) and then kept in a shaker for extraction at room temperature for 24 h. Then, methanol was dissipated from the sample using oven to get the crude extract which was re-suspended in dimethylsulfoxide (DMSO) for application in the antibacterial test.

Lipid Extraction from Leaves

Around 470g of the fresh leaves with 1 L of distilled water were grinded by a blender. The solution was mixed with the solvent (chloroform: methanol in 2:1 ratio) to separate the lipids and then evaporated in a rotary evaporator. The extract was filtered through charcoal. The obtained oil (1 g) was kept in storage at 4°C until utilization for further tests.

Anti-bacterial Assay

Muller Hinton Agar CM0337 from Oxiod (Part of Thermo Fisher Scientific) was used in well diffusion assay. MHA contains beef dehydrated infusion 300.0 g/L, casein hydrolysate 17.5 g/L, starch 1.5 g/L and agar 17.0 g/L. Amount of 38 g of MHA was suspended in 1 L of distilled water, boiled and sterilized by autoclaving at 121°C for 15 min.

Three strains of pathogenic bacteria *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853, and *S. aureus* ATCC 25923 were utilized as test microorganisms. These clinical isolates were obtained from Microbiology Laboratory at Sultan Qaboos University Hospital. These strains are for antibiotic testing and fall under the American type collection culture (ATCC). Furthermore, these strains were sub cultured in liquid broth for a period of 6 to 8 hours. The well diffusion assay was conducted utilizing Muller Hinton Agar (MHA). The assay of leaves, stem and roots extract activity was carried out in nine MHAs plates which were replicated three times. For every plate, four discs were used one each for leaves, stem, roots and an antibiotic standard (Ciprofloxacin) as positive control. Discs of 6 - 8 mm diameter were removed from agar with a sterile glass pasture pipette and filled with 30 µl of the sample extract or standard. At the same time, three MHAs plates were used for oil using a similar procedure where three discs were used for every plate; two discs for oil and one disc for antibiotic standard. Zone inhibition was investigated after incubating agar plates at 37°C.

Sample Preparation and Extraction for GC-MS Analysis

Fresh leaf, stem and root samples were weighed and grinded using a mechanical grinder. Then, 50 mL of 70% methanol was added to each grinded sample and placed in an ultrasonic water bath working at 50–60 kHz with power of 350 W for 30 min at room temperature. By using a rotary vacuum evaporator, the methanol was evaporated and the extracts were concentrated. The crude extracts were dissolved in hexane, filtered by microfiltration (0.45 µl syringe) and injected to GC-MS.

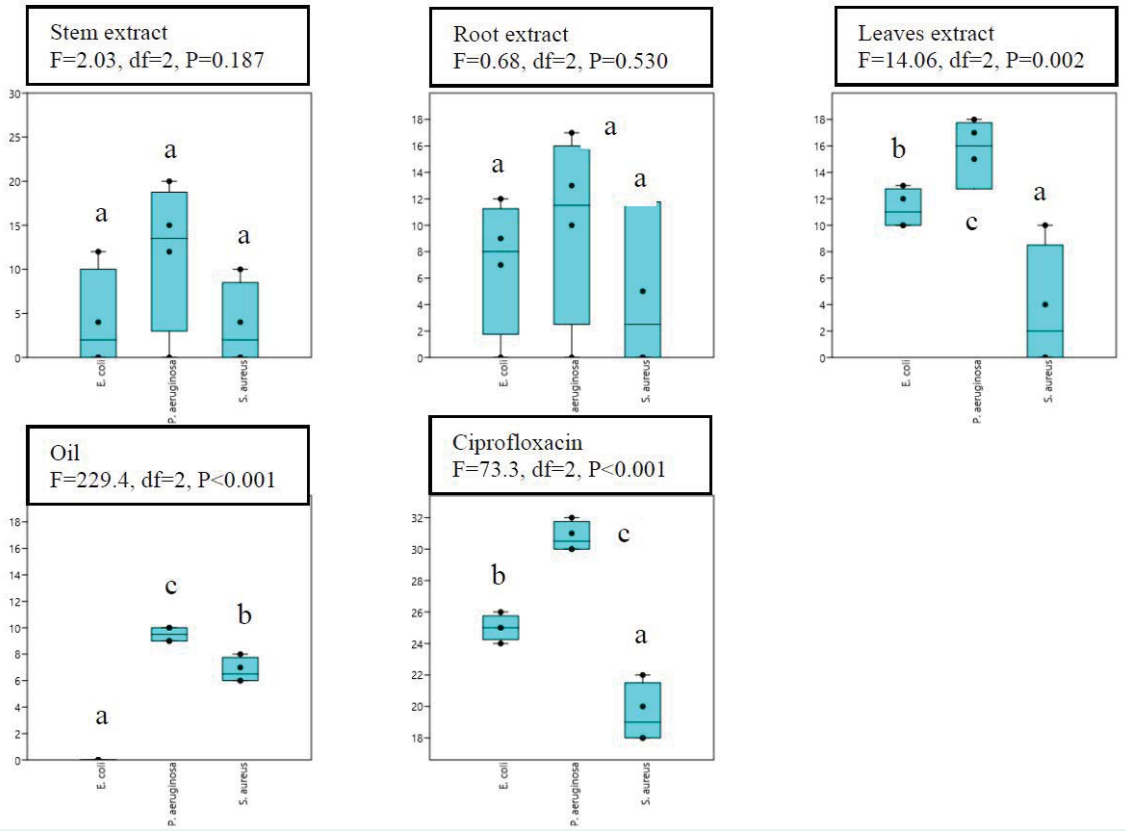

Figure 1. Comparison of anti-bacterial activity (inhibition zone in mm) among *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923 and *Pseudomonas aeruginosa* ATCC 27853 in well diffusion assays exposed to *Zygophyllum luntii* extracts and Ciprofloxacin. Bars designated by the same letters are not statistically significant at $\alpha_{0.05}$

Gas Chromatography-Mass Spectrometry (GC/MS) Analysis

GC-MS conditions for samples extracted from leaves, stem and roots: GC-MS analysis was performed on a Perkin Elmer Clarus 600 GC System, fitted with a Rtx-5MS capillary column (30 m × 0.25 mm i.d. × 0.25 µm film thickness; maximum temperature, 350°C), coupled to a Perkin Elmer Clarus 600C MS. Ultra-high puri-

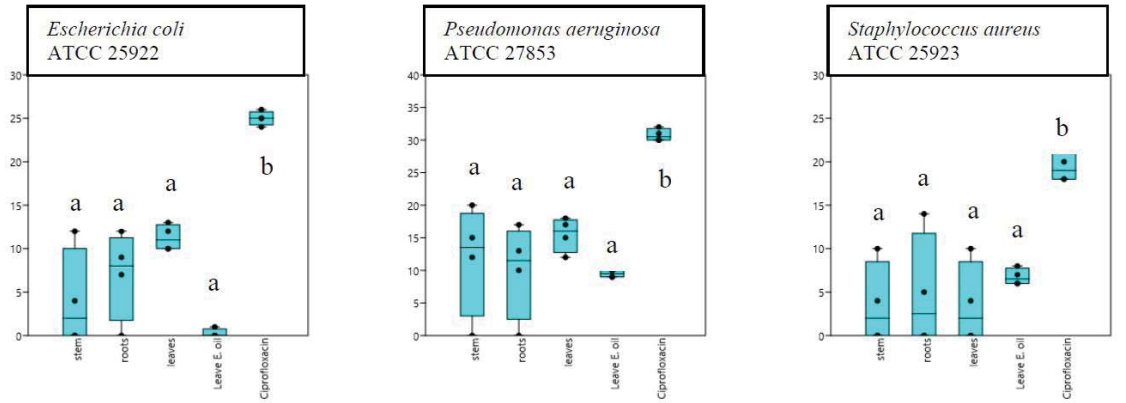

Figure 2. Comparison of anti-bacterial activity (inhibition zone in mm) of the *Zygophyllum luntii* extracts and Ciprofloxacin against *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and *Staphylococcus aureus* ATCC 25923 well diffusion assays. Bars designated by the same letters are not statistically significant at $\alpha_{0.05}$

Table 1. Compounds identified in the leaf, stem and root extracts of *Zygophyllum luntii* by GC-MS

Name of compound	Retention Time (min)	Percent (%)
Leaves		
Pentadecane	13.64	0.33
Tetradecane, 2,6,10-trimethyl-	16.43	0.68
Oxalic acid, allyl octyl ester	16.81	0.32
Unidentified	17.02	0.61
Octadecane	17.79	0.34
Nonadecane	18.73	0.90
Eicosane	19.02	5.84
Heneicosane	21.57	12.30
Docosane	22.60	12.72
Tricosane	23.26	12.93
Tetracosane	24.24	9.94
Pentacosane	25.36	10.42
Hexacosane	26.21	7.63
Heptacosane	27.13	5.84
Octacosane	27.87	5.82
Nonacosane	28.70	5.03
Triacontane	29.62	4.10
Hentriacontane	30.71	1.84
Dotriacontane	31.99	2.42
Tetratriacontane	36.24	0.13
Stem		
Hexadecanal	18.33	2.60
Phytol	21.50	5.22
Unidentified	22.22	2.70
Unidentified	23.20	0.38
Unidentified	24.20	0.27
Unidentified	25.18	0.47
Heptacosane	26.90	0.35
Octacosane	27.80	0.22
Supraene	28.14	0.95
Unidentified	28.60	0.46
Triacontane	29.50	0.29
Hentriacontane	30.64	0.38
Vitamin E acetate	31.40	1.24
Dotriacontane	31.93	0.64
Tritriacontane	33.40	1.23
β-Sitosterol	34.36	11.02
Heptatriacontane	35.40	0.80
Hop-22(29)-En-3.Beta.-Ol	35.90	69.95
Octatriacontane	37.70	0.84
Roots		
Docosane	22.60	14.04
Tricosane	23.49	14.36
Tetracosane	24.43	12.54
Pentacosane	25.36	4.02
Pentacosane	25.54	5.33
Hexacosane	26.24	6.67
Octacosane	27.13	43.05
Tetratriacontane	36.24	1.15

ty helium (99.9999%) was used as carrier gas at a constant flow of 1.0 mL/min. The injector, transfer line and ion source temperatures were 280°C, 270°C and 270°C, respectively. The ionizing energy was 70 eV. Electron multiplier (EM) voltage was obtained from auto tune. All data were obtained by collecting the full-scan mass spectra within the scan range 40-550 amu. The injected sample volume was 1 µl with a split ratio of 10:1. The oven temperature program was 60°C at a rate of 80°C per minute to 280°C hold for 25 minutes. The total run time was 53.5 minutes.

GC/MS conditions for the oil extracted from leaves: Fatty Acid Methyl Ester (FAME) compounds were detected by the GC-MS equipment as mentioned above. The helium gas flow rate was 0.7 mL/min. The injector, transfer line and ion source temperatures were 250°C, 250°C and 220°C, respectively. The initial oven temperature was set at 50°C (holds for 8 minutes) and increased to 250°C in a rate of 40°C per minute. All data were obtained by collecting the full-scan mass spectra within the scan range 35-500 amu. The unknown compounds were identified by comparing the spectra obtained with mass spectrum libraries (NIST 2011 v.2.3 and Wiley, 9th edition).

Statistical Analysis

Data on antibacterial assays from leaves, stems and roots, and the three bacterial strains were analysed separately using one-way analysis of variance (ANOVA) in Past (https://past.en.lo4d.com/windows). Means were separated by Dunn's Multiple Comparison Test and differences were considered significant at $p<0.05$.

Table 2. Compounds detected in the oil extracted from *Zygophyllum luntii* leaves by GC-MS

Name of compound	Retention Time (min)	Percent (%)
	Leaves	
Caprylic acid methyl ester	11.58	0.12
Capric acid methyl ester	18.27	0.19
Lauric acid, methyl ester	24.29	0.74
Myristic acid, methyl ester	29.83	0.81
Palmitic acid, methyl ester	34.93	34.55
Palmitoleic acid, methyl ester	35.90	1.70
Oleic acid, methyl ester	39.78	23.99
Linoleic acid, methyl ester	40.57	5.81
Linolenic acid, methyl ester	41.73	32.09

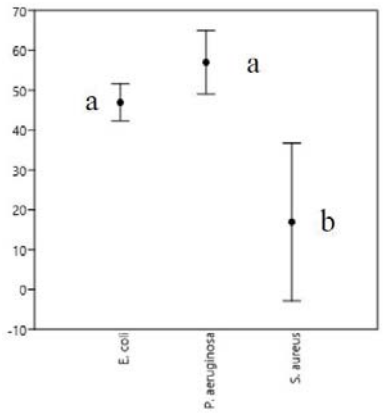

Figure 3. Percent effectiveness of the *Zygophyllum luntii* extracts compared to Ciprofloxacin against *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923 and *Pseudomonas aeruginosa* ATCC 27853, and *S. aureus* well diffusion assays. Bars designated by the same letters are not statistically significant at $\alpha_{0.05}$

Result and Discussion

Antibacterial Assays

Significant antibacterial activity was observed in the *Z. luntii* leaves extract and significant differences (F=14.06, df=2, P=0.002) were found among *E. coli*, *P. aeruginosa* and *S. aureus* (Figure 1). The highest inhibition was observed against *P. aeruginosa* (15.5 ± 2.64 mm) then *E. coli* (11.25 ± 1.50mm) and *S. aureus* (3.50±4.72 mm). The inhibition zones of the stem (F=2.03, df=2, P=0.187) and root (F=0.68, df=2, P=0.530) extracts did not differ significantly among the three bacterial strains. Oil from leaves did not produce any inhibition in *E. coli* but had significantly higher inhibition (F=229.4, df=2, P<0.001) in *P. aeruginosa* (9.5±0.58mm) and *S. aureus* (6.75±0.96mm). Ciprofloxacin had significantly different inhibition zones (F=73.3, df=2, P<0.001) among the three bacterial strains. The highest inhibition zone by the commercial antibiotic was against *P. aeruginosa* (30.75± 0.96 mm) then *E. coli* of (25.0± 0.82 mm) and lowest against *S. aureus* (19.50±1.91 mm).

The inhibition in the stem, root and leaves extracts, and oil from leave was significantly lower than Ciprofloxacin against *E. coli* (F=29.8, df=4, P<0.001), *P. aeruginosa* (F=11.7, df=4, P<0.001) and *S. aureus* (F=9.9, df=4, P<0.001) (Figure 2). The stem extract was more active against *P. aeruginosa* while root extract was more active against *S. aureus*. Leaves extract showed more activity against *E. coli*. The oil showed some antibacterial activity against *P. aeruginosa* and *S. aureus* but not *E. coli*. Overall, the *Z. luntii* extracts were active against both Gram-positive (*S. aureus*) and Gram-negative bacteria (*E. coli* and *P. aeruginosa*), though they were more active against the latter. The ethanol extracts of intact leaf of *Z. arabica* showed an inhibition zone of only 6.08 mm against *E. coli* and did not inhibit the growth of *S. aureus* (Alam et al., 2010). The ethanol whole plant extract of *F. cretica* produced inhibition zones of 15 mm, 15 mm and 14 mm against *E. coli*, *P. aeruginosa* and *S. aureus*, respectively (Sajid et al., 2011) which are similar to our results. The crude extract of *Z. arabica* from Sinai showed broad antimicrobial spectrum against Gram-positive, Gram-negative, spore-forming and acid-fast bacteria (El-Hefnawi, 1999). It is obvious that leaves extracts from *Zygophyllum* spp including *Z. luntii* have antibacterial properties.

The percent effectiveness of the *Z. luntii* extracts were compared to Ciprofloxacin against *E. coli*, *P. aeruginosa* and *S. aureus* by subtracting the extract inhibition zone sizes from the Ciprofloxacin (Figure 3). The calculated percent effectiveness against *E. coli* was 47.0±4.8%; against *P. aeruginosa* was 57.1±8.1%; and against *S. aureus* was 19.4±24.2%. The percent effectiveness against *E. coli* and *P. aeruginosa* was significantly higher compared to *S. aureus* (F=10.54, df=2, P=0.005). Alam et al. (2010) produced callus of *Z. arabica* by tissue culture and found that the callus extract was more effective against *Serratia marcescens*, *E. coli* and *Acetobacter aceti* subsp. liquefaciens (inhibition zones = 32.67, 33.92 and 34.83 mm respectively) than the crude extract of the intact leaf (IZ=6.08 mm) suggesting higher antibacterial effects of callus extract against Gram – ve bacteria.

Several pathogens are increasingly developing resistance, particularly to broad-spectrum antibiotics (Kunin, 1993). Resistant *E. coli* isolates have been reported from humans using disk diffusion method against ciprofloxacin (22% with the highest of 52% reported from Iran), cefotaxime (31.2%–58%) and ceftazidime (10%–57.4%) (Pormohammad et al., 2019). Some of the gram-positive drug resistant bacteria include *S. aureus*, *Streptococcus*

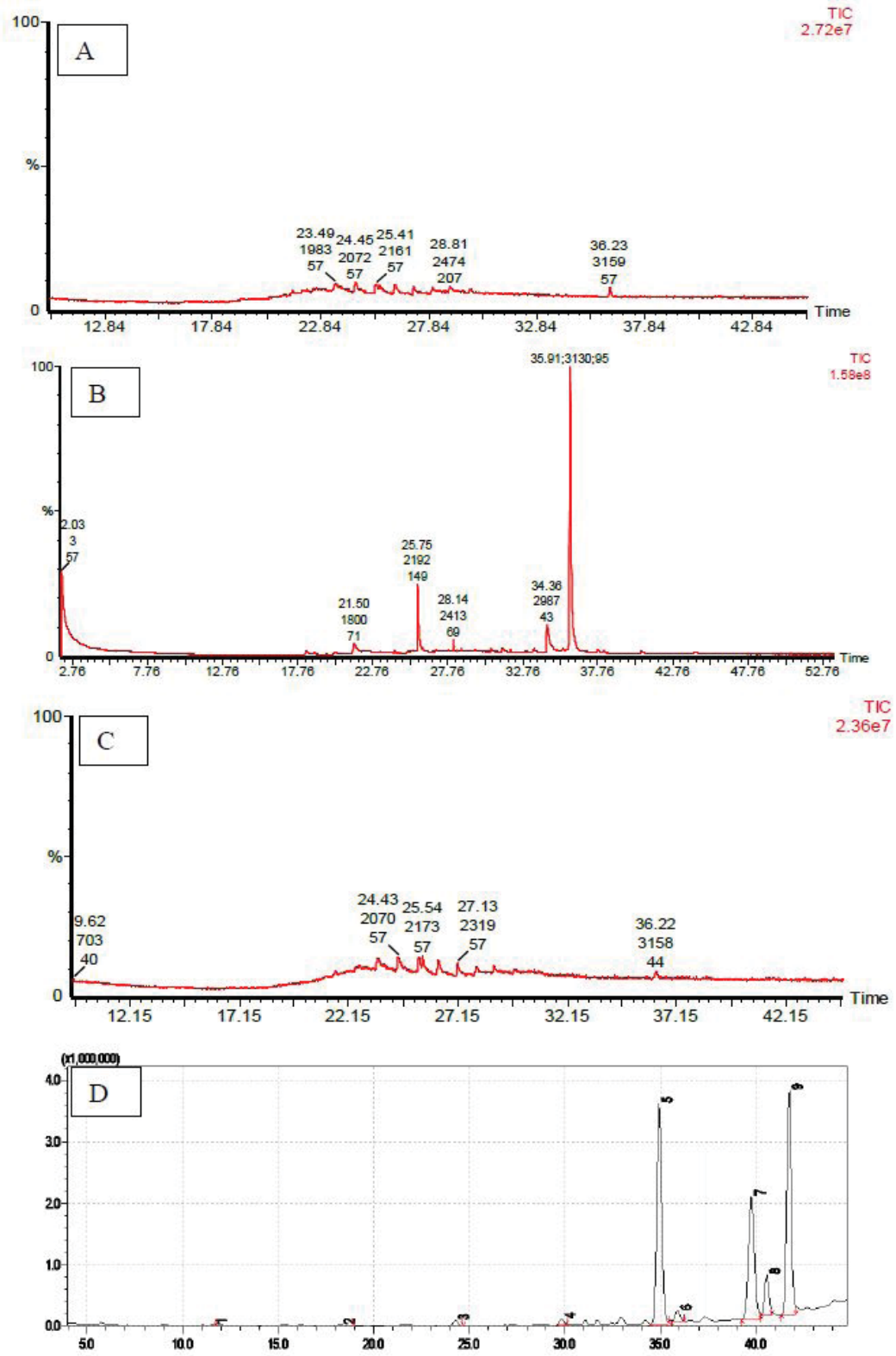

Figure 4. Chromatogram of compounds in leaves (A), stem (B) and roots (C), and fatty acids methyl esters (FAME) in oil (D) extracted from *Zygophyllum luntii* leaves detected by GC/MS.

pneumoniae, and *Enterococcus* spp., and the gram-negative drug resistant bacteria *Acinetobacter baumannii*, *Klebsiella pneumoniae*, *E. coli,* and *P. aeruginosa* (Lister et al., 2009). Our results also indicated reduced sensitivity of *S. aureus* and *E. coli* to Ciprofloxacin. The *Z. luntii* callus extract may help in managing antibacterial resistant pathogens of different strains

Gas Chromatography-Mass Spectrometry (GC/MS) Analysis

Higher number of compounds was identified in the leaves (n=20) and stem (n=19) extract compared to roots (n=8) extracts of *Z. luntii* while 6 compounds could not be identified (Table 1). The retention time (RT) of all compounds varied between 13.64 to 37.7 minutes. The unidentified compounds were present in a relatively low amount. Heneicosane, Docosane and Tricosane were present in higher quantities in the leaves extract. Hop-22(29)-En-3.Beta.-Ol (69.95%) was present in higher quantities in the stem extract while the root extract had octacosane in high quantities. Palmitic acid, linolenic acid and oleic acid were the main components of oil extracted from leaves (Table 2).

Heptacosane, Heneicosane, Tetradecane have been reported with antimicrobial activity (Elshiekh and Abdelmageed, 2015). Beta-acids are an important component of hops (*Humulus lupulus* L. family Cannabaceae) soft resins and usually isolated as by-products during hop processing (McCallum et al., 2019). Hexahydro-β acids showed strong antibacterial activity and good stability (Liu et al., 2019). Noticeably, HOP-22(29)-EN-3. BETA.-OL had the highest percentage compared to other compounds.

Close retention time of 22-27 minutes in roots compounds showed their similar affinity to stationary phase. Both retention time and peak area (%) for Docosane (C22) and Tricosane (C23) (22.60 minutes and 23.26 minutes, and 12.72% and 12.93%, respectively) did not show wide variation. These two compounds were present in leaves and roots but not in stems. The n-alkane fractions (hydrocarbons C22-C35) was detected in the leaves extract of *Z. luntii* which have been detected in vegetable oils by GC/MS (Troya et al., 2015). These compounds are more common in plant extracts.

Species of *Zygophyllum* have been found to contain saponins (Abdel-Khalik et al., 2001), alkaloids (Sharawy and Alshammari, 2009), terpenoids (Perroni et al., 2007), sterols (Shoeb et al., 1994), flavonoids (Ibrahim et al., 2008), proteins and amino acids (Sharma et al., 2010), coumarins (Alam et al., 2010) and trace elements (Fatima et al., 1999). The presence of such chemical ingredients in *Zygophyllum spp*. Would contribute to the medical properties, including stimulating the immune system in humans, treating and preventing the development of chronic diseases (Beier, 2005), and the vitality to resist such types of pathogenic bacteria used in this study.

Oil Extract from Leaves

Nine different types of fatty acids methyl esters (FAME) were found in oil extracted from *Z. luntii* leaves. Palmitic acid, Linolenic acid, and Oleic acid were the main compounds and represented 90% of the oil (Fig.4). There was no study found on *Z. luntii* that explored the fatty acid content. However, seven fatty acids including the Oleic acid, Palmitic acid and Linoleic acid were found in other *Zygophyllum* species e.g. *F. arabica* L (Alam et al., 2010) and *F. cretica* (Soad, 1994).

Conclusion

Extracts from leaves, stem, and roots of *Z. luntii* had significant antibacterial activity against *E. coli*, *P. aeruginosa* and *S. aureus*. The extracted oils had activity against only *P. aeruginosa* and *S. aureus*. The commercial antibiotic Ciprofloxacin had reduced activity against *S. aureus* and *E. coli*. The *Z. luntii* extracts showed about 50-60% effectiveness against *E. coli* and *P. aeruginosa* compared to Ciprofloxacin. HOP-22(29)-EN-3. BETA.-OL, Hexahydro-β acids, and FAME compounds could have contributed to the antibacterial activity. With further research on callus production, improving extraction process and antimicrobial activity assays against more pathogenic bacterial (including antibiotic resistant) species, *Z. luntii* can be promoted as a source of traditional medicine in Oman

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The editorial board recommends the use of a classic typeface (Times, Helvetica, Garamond, Myriad-Pro, …) with 12 point size and at least 14-16 points leading (line spacing) for the text of the manuscript with minimum formatting as most of layout and typographical formats will be applied at the typesetting stage. The manuscript page size should be A4 or US Letter and the editorial board recommends that a margin of at least 3 cm be included on all sides of the paper.

To facilitate the reviewing process, the text of the submission should have line numbers in the left hand margin restarting at 1 on each page and a page number in the footer of the document. Use standard typographic conventions for the text presentation: italic typeface for species names (not underline), bold face for vectors, true superscript and subscripts when necessary. Emphasis is better marked through italic rather than bold face.

Typography

The journal uses standard typographic convention throughout. The editorial board recommends that you follow these in the preparation of the manuscript.

Italic should be used throughout for the Latin name of species (please do not use underlined text). Emphasis can be placed on some elements of text using bold face.

Abbreviations: Avoid non-standard abbreviations whenever possible, particularly in headings and subheading. If, for the sake of

conciseness, the author wishes to use abbreviations, define each abbreviation when they first appear in each section of the manuscript. Standard abbreviation such as RNA, DNA, ATP, ADP, EDTA… do not need to be defined as most readers will be familiar with them. Others such as PAH (Polycyclic Aromatic Hydrocarbon) or ICP (Inductively Coupled Plasma) should be defined as most reader may not be familiar with their meaning.

Units: Always use the International System of Units (SI) for all units. For large or small units use the standard multiplier prefix for the units (k for 1000, M for 1000000, m for 1/1000 and µ for 10-6). Prefer whenever possible negative exponents to slash: kg·m-2 rather than kg/m2. To separate units, use either a mid-line point (· =ASCII code 183 – Unicode U+00B7) or a non-breaking space. The abbreviated symbols (k, kg, s, P, W, etc.) should be used whenever possible and combined with Arabic numbers (5 kg, 2 m2, 5.2 MP, 6.78 MW·h). The only exception is when a number is grammatically placed at the beginning of the sentence. A non-breaking space (Unicode U+00A0) should be used between the number and its units to insure that they stay together in the final document. The SI unit of time is s (second), h stands for hora, min for minuta, d for dies (day) and a for annum (year).

When necessary, non-SI units can be added between parenthesis to allow comparison with older literature or traditional systems of measurements. This includes usual units, such as surface of farming units (faddan, acres, ares, hectares), or traditional depth units (fathoms, brasses, Ba',…) or distance (nautical miles, miles) or other non SI units (gallons, inch, foot, bushels, etc.).

Illustrations

Illustrations should be numbered consecutively and submitted as individual files, not embedded in the article file. To insure compatibility, the journal accepts the following file format: JPEG, TIFF, PNG, PDF, EPS and SVG. Although the journal is normally published in black and white, color illustrations can be used when color is clearly necessary to convey the intended message. Although the authors can suggest the inclusion of color figures in the paper, the final decision to include them or not is left to the editorial board. The editorial team will strive to provide the best possible graphic output from the material submitted by the authors and may in some cases decide to redraw some figures to improve readability. They may also request better quality photographs or color figures if necessary. Typically black and white line figures should have a resolution of at least 600 dpi (at the final printed size) and color figures or photographs 300 dpi (at the final printed size) but should not exceed 10 MB. For line graphics, vector based file formats (SVG, PDF, EPS) are preferred as they are resolution independent.

Each illustration should have at the bottom of the page a brief identifier such as the name of the first author, the word Figure and the sequential number of the figure. (Al-Oufi, Figure 7 for instance). The full captions of all figures should be presented in numerical order on a separate page at the end of the text manuscript.

In the figure use Helvetica as the standard typeface for all text (axis, legend, axis legend, equations, labels, etc.) and ensure that all text remain legible even after size reduction for final printing. Figures will be printed either as a single column (7 cm wide) or double column (14 cm) figure

Macro-photographs, micro-photograhs, SEM photographs, anatomical drawings, morphological illustrations, should have an appropriately labeled scale bar. Avoid multiplication factors (x100, x10000) as these will change with the rescaling of the figure when printed.

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Tables should be presented in a clear manner and designed to fit on the width of a page. Exceptionally wide tables may be typeset, sideways, along the height of a printed page. All unnecessary decimals should be removed. Tables should be included at the end of the manuscript on separate pages and the legend/caption of each table should be placed on the same page and above the table.

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Equations should be placed on separate lines and numbered sequentially at the end of the line. They should be typeset using an equation editor. If this is not possible scan or photograph a clear handwritten version of the equation which will be typeset by the editorial team.

The Journal uses the modern scientific number styles recommended by the Council of Science Editors. This styles uses digit numbers (1, 2,4.5, 7, etc.) for all numeric representations, even single digit ones. The main exceptions are when a digit starts a sentence, or when the single digit number is part of an idiomatic expression such as in "one or both", a "zero-tolerance policy", a "one-to-one interview", "one has to agree that"…

References

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Jones et al. (2007) listed 4 main types of …

According to Jones et al. (2007), the main reason for….

The prevalence of coral parasites was considerably lower than that reported in Tanzanian coral reefs (Mwaniki, 1996).

Different papers that share the same in-text citation format (same authors and same year of publication) are identified by a small letter (a, b, c) following the date of publication. When used in a single citation, the author's name is not repeated.

The ticks collected on camels were not different from that found on goats (Bobade, 2004a) or sheep (Bobade, 2004b).

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

The family name of the first author is always used. For publications with 2 authors, the 2 family names with the conjunction "and" are used followed by the year of publication. For more than 2 authors, the abbreviation et al. (Latin et alii – and others) is used to replace all but the first author's name.

Jones et al. (2007) listed 4 main types of soft coral communities…

Al-Barwani and Jones (2005) found 3 genetically distinct populations of mussels…

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

End of text references

The bibliographic information for all cited references in the articles are listed at the end of the papers under the heading "References". The list of references is sorted first following the alphabetical order of the authors and if necessary, by the date of publication. Please follow the examples shown below, including punctuation. The Journal names follow a "Title case" capitalization—all words are capitalized except for articles (a, an, the); for prepositions (against, of, in, to), for conjunctions (and, for, not, or)—and should NOT be abbreviated. Titles of articles, books, on the other hand follow a

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Adams NA. (2001). UV radiation evokes negative phototaxis and covering behavior in the sea urchin *Strongylocentrotus droebachiensis*. Marine Ecology Progress Series 213: 87-95.

Journal reference with 2 authors

Zhou M, Huntley ME. (1997). Population dynamics theory of plankton based on biomass spectra. Marine Ecology Progress Series 159: 61-73.

Journal reference with more than 2 authors

Schlacher TA, Thompson I, Price S. (2007). Vehicles versus conservation of invertebrates on sandy beaches: Mortalities inflicted by off-road vehicles on ghost crabs. Marine Ecology 28: 354-367.

Whole book

Parsons TR, Maita Y, Lalli CM. (1984). A manual of chemical and biological methods for seawater analysis. New York: Pergamon Press. 173 pp.

Book chapter from an edited book

Brooks HA, Probert TH. (1984). Let's ask GMDH what effect the environment has on fisheries. In: Farlow SJ, editor. Self-organizing methods in modeling. Gmdh type algorithms. New York and Basel: Marcel Dekker, Inc. p.169-178.

Report

Stransky C. (2001). Preliminary results of a shape analysis of redfish otoliths: Comparison of areas and species. Northwest Atlantic Fisheries Organization. NAFO SCR No. 4382.

Thesis

Al-Masroori HS. (2002). Trap ghost fishing problem in the area between Muscat and Barka (Sultanate of Oman); an evaluation study [MSc.]. [Muscat]: Sultan Qaboos University. 112 pp.

Article not in English

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.

Conference proceedings

Campbell AC. (1988). The echinoderm fauna of Dhofar (southern Oman) excluding holothuroids. In: Burke RD, Mladenov PV, Lambert P, editors. Proceedings of the Sixth International Echinoderm Conference; 23-28 August 1987; Victoria, Canada: Balkema. p. 369-378.

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1. The current submission has not been previously published nor is it currently submitted to another journal for consideration.

2. The submission text files are in Microsoft Office (.doc, .docx), OpenOffice (.odt), RTF (rtf) or Apple Pages (.pages) document file format.

3. The text of the document uses a 12-point standard font with a 14-16 point leading (space between lines) on A4 or US-Letter format pages with page numbers and line numbers. Manuscript conforms to the journal recommended styles, length and number of sections.

4. The Abstract of the paper follows the structured format described in the guide for authors and includes a single paragraph (<300 words) with 5 inline headings (Introduction, Objectives, Method, Results and Conclusions) and keywords for the manuscript are provided.

5. Photography (or photographic plates) are submitted in the jpeg (.jpg) file format at 300 dots per inch (dpi) with 80% compression quality or better. Line drawings and other figures should be preferably submitted as vector graphics such as pdf, eps or svg files. Alternatively, high resolution (600dpi) image format are acceptable (PNG, TIFF, GIF).

6. All tables including (legend, description and footnotes) and all figure captions are part of the submission main text file.

7. The text adheres to the stylistic and bibliographic requirements outlined in this document which can also be found in About the Journal on the Journal web site.

8. The manuscript has been "spell-checked" and "grammar-checked".

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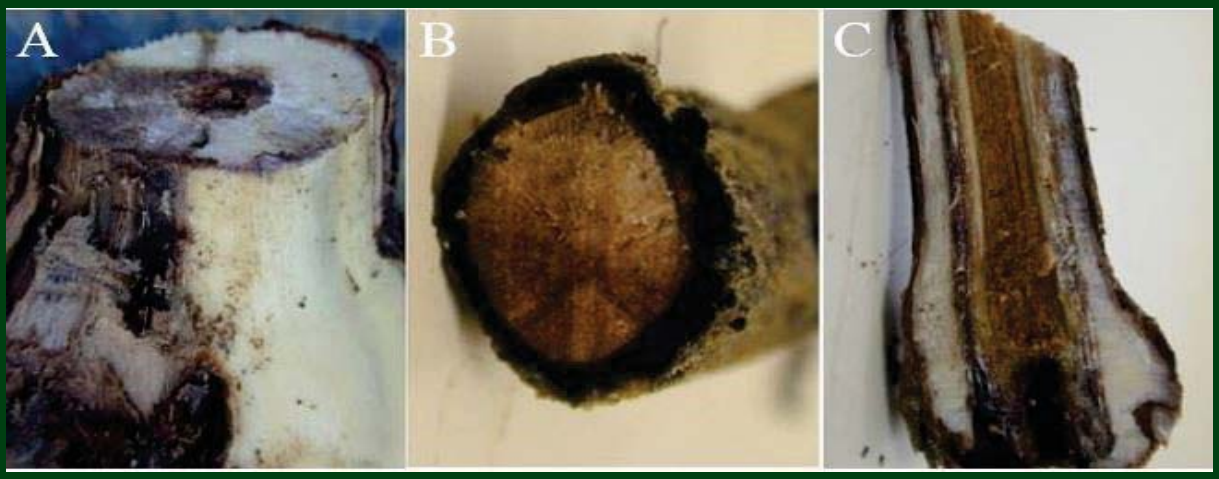

- The cross-section of rootstock infected by black foot disease (A), cross-section of the root (B) and longitudinal-section of a young grapevine infected with *Cylindrocarpon* spp. (C) [adapted with the permission from Halleen et al. (2007)].

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المجلد ٢٥، العدد ٢، ٢٠٢٠