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Preliminary assessment of insecticidal activity of Moroccan actinobacteria isolates against mediterranean fruit fly (*Ceratitis capitata*)

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Microbial insecticides are considered as the most sustainable and ecologically acceptable means of crop protection. Here we report the ability of some Moroccan actinobacteria isolates to produce larvicidal compounds against the Medfly (*Ceratitis capitata* Wied.). Thus, actinobacteria isolates were tested for their insecticidal activity through biological and chemical screening. The primary biological screening using the brine shrimp bioassay showed that 12 isolates out of 210 (5.71%) have been found to be highly toxic at the concentration of 100 µg mL^{-1}. Among these, isolates OS46, 37 and B62 were the most toxic with an LC_{50} of 0.26, 0.34 and 0.84 µg mL^{-1}, respectively. The freeze-dried fermentation of the selected isolates showed moderate to high insecticidal activity against the first instar larvae of *C. capitata*, and the most important pupation reduction was obtained for isolates 37 and B89 (0 and 6% of pupation, respectively). The chemical screening on thin layer chromatography of the crude extract of the most important isolates using specific insecticide family's reagents showed a variety of compounds depending on isolate with at least one active spot for each tested specific reagent. Finally, the inhibition of acetylcholinesterase activity test was carried out in order to assess the possible nature of insecticidal activity of selected isolates. The obtained results show that, except the isolate 37 which probably acts by a different mode of action, all other isolates were active. This finding could have an applicative value for the potentiality of utilizing Moroccan actinobacteria isolates as an alternative to chemical insecticides in pest management mainly against *C. capitata*.

Key words: Moroccan actinobacteria, insecticidal activity, biological screening, chemical screening, *Ceratitis capitata*.

INTRODUCTION

With an ever-increasing of human population, the demand placed upon the agriculture sector to supply more food is one of the greatest challenges for the agrarian community. An increased yield potential of

crops, however, is often constrained by different pest attacks (Oerke and Dehne, 2004). In fact, an average of 35% of global crop production is lost due to insect pest (Oerke, 2005). A case in hand concerns one of the most economically costly pest species worldwide; the Mediterranean fruit fly, *Ceratitis capitata* (Weidemann) (Diptera: Tephritidae) (Malacrida et al., 2007; De Meyer et al., 2008). This species shows a wide larval hosts range comprising more than 200 different species of fruits and vegetables (Lance et al., 2014). In Morocco, the Medfly survives in large forests (800,000 hectares) of endemic Argan trees and invades continuously bordering agricultural areas (Alaoui et al., 2010). The control of the Medfly has been mostly done with chemical insecticides (especially malathion) (Bolognesi, 2003, Dominiak and Ekman, 2013). However, their intensive use and abuse have led to several problems such as the pollution of the environment (Kumari et al. 2008; Ferencz and Balog, 2010), an increase in human diseases such as cancer and several immune system disorders (Osman, 2011), the selection of insect resistant populations (Rivero et al., 2010), and important outbreaks of secondary pests (Croft, 1990; Ruberson et al., 1998; Preetha et al., 2010).

Microbial insecticides have been recognized as an important alternative to the use for pest control (Ravensberg, 2015). Unlike most chemical insecticides, bioinsecticides are often very specific for a particular pest. They have less impact on the environment and water quality, and they offer more environmentally friendly alternative to chemical insecticides. They could also be used where pests have developed resistance to conventional pesticides (Popp et al., 2013). For these reasons, there is an increasing concern in society for lowering the use of chemical insecticides and moving to safer practices in crop protection (Jemâa et al., 2010 ; Chueca et al., 2013), such as the development of new environmental friendly microbial insecticides (Aboussaid et al., 2011). Among bacteria, actinobacteria are the most prolific source for all types of bioactive metabolites, including bioinsecticides (Berdy, 2005; Liu et al., 2008; Herbert, 2010; Karthik et al., 2011; Omura, 2011; Deepika et al., 2012; Saurav et al., 2013). Currently, the control of *C. capitata* by actinobacterial insecticide is based on spinosad (Barry et al., 2003; Vargas et al., 2003; Stark et al., 2004). However, in laboratory experiments, some pest insects have developed resistance to this insecticide (Hsu and Feng, 2006; Su and Cheng, 2013 ; Abbas et al., 2014). Thus, the use of others actinobacteria as a biocontrol agent against the Medfly could ensure environmental protection and commercial sustainability.

The present study has been undertaken to select Moroccan actinobacteria isolates with insecticidal activity using a primary biological screening against *Artemia salina,* successfully used as a model for biological screening of insecticidal activity (Xiong et al., 2004 ; Shiomi et al., 2005; Deng et al., 2008), and a secondary biological screening against the first instar larvae of *C. capitata* (Wiedemann). Finally, the chemical screening through specific insecticide reagent was done in order to assess the possible nature of insecticidal activity of selected isolates.

MATERIALS AND METHODS

Microorganisms

The 210 actinobacteria isolates used in this study were from the collection of Laboratory of Biology and Biotechnology of Microorganisms, Cadi Ayyad University, Marrakesh, Morocco. They were isolated from various Moroccan habitats including rhizospheric soils and endophytic of endemic aromatic and medicinal plants (Barakate et al., 2002). All strains were maintained in glycerol (20%) at -20°C.

Fermentation

Selected actinobacteria isolates were inoculated into a 500 mL baffled Erlenmeyer flasks containing 100 mL of the producing Bennett's liquid medium (Beef extract, 1 g L^{-1} ; glucose 10 g L^{-1} ; peptone, 2 g L^{-1} ; yeast extract, 1 g L^{-1} ; distilled water, 1000 mL ; pH 7.2). Flasks were incubated on a rotary shaker (250 rev min^{-1}) at 30°C for 48 h. 2 L of this culture was used as inoculum for a 20 L jar fermentor containing 18 L of the Bennett's liquid medium. Starting pH is at about 7.2 and the aeration was of 5 L min^{-1} with agitation of 120 rev min^{-1}. The fermentation was carried out at 30°C for at least seven days. The bacterial culture received from the fermentation was first filtered over the Celite and the water phase was in part freeze-dried and used for larvicidal bioassay of *C. capitata*. The remaining liquid fermentation was extracted three times with acetic ester with a ratio of 1:1 (v/v). The organic phases were collected together and evaporated in vacuum at 40°C until they became dry. The obtained crude extract was suspended in methanol for further uses in chemical screening and acetylcholine-esterase test inhibition.

Primary screening: Brine shrimp bioassay

The brine shrimp lethality assay is considered as a useful and effective tool for preliminary screening of insecticidal substances of microbial origin (Liou, 2000 ; Zhiyu et al., 2000). Our primary biological screening's brine shrimp bioassay was conducted using agar culture diffusion solution according to the method of (Xiong et al., 2004) with some modifications. The agar culture diffusion solution was prepared as follows: actinobacteria isolates were grown on Bennett's agar medium (Beef extract, 1 g L^{-1} ; glucose, 10 g L^{-1} ; peptone, 2 g L^{-1} ; yeast extract, 1 g L^{-1} ;agar, 15 g L^{-1} ; distilled water, 1000 mL ; pH 7.2) and incubated for at least 7 days at 30°C. The agar culture diffusion solution was prepared by picking out under sterile conditions 1 cm^2 of agar culture of the isolates and

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put into Eppendorf tubes containing 1 mL of sterile distilled water. The tubes were kept at 4°C for 12 h. The mixtures were centrifuged at 1000 g for 15 min and the supernatants were pipetted out. Brine shrimp eggs (0.5 g) were hatched in 500 mL sterile seawater. The suspension was aerated by bubbling air and kept for 24 to 48 h at room temperature. A volume of 0.5 mL of hatched nauplii suspension (30 to 40 larvae) was transferred into separate wells of a plastic 24-well tissue culture plate and filled with 0.5 mL of each actinobacteria supernatant. The supernatant obtained from a free isolates agar culture was used as negative control. All experiments were conducted in six replicates. The mortality was adjusted using the Abbott's control-adjusted mortality (Abbott, 1925) expressed as follow :

$$M = \frac{(A-B-N)}{(G-N)} \times 100$$

Where, M = percent of the dead larvae after 24 h; A = number of the dead larvae after 24 h; B = average number of the dead larvae in the blind samples after 24 h; N = Number of the dead larvae before starting the test and G = Total number of larvae.

For the isolates showing a strong anti-brine shrimp activity, the LC_{50} and TL_{50} were calculated.

Secondary screening: Laboratory bioassay of *C. capitata*

Insect rearing

C. capitata used in these tests was obtained from a mass-reared stock maintained at the laboratory of Molecular modeling and Ecophysiology, University Cadi Ayyad, Faculty of Sciences Semlalia, Marrakesh, Morocco (Aboussaid et al., 2011). The flies were maintained and all the experiments were carried out at 27 ± 2°C and 75 to 85% relative humidity under 16:8 light and dark cycles. Insects were reared in clear plastic containers (20 × 14 × 7 cm) on a diet composed of wheat bran, sucrose, brewer's yeast, Nipagin, Nipasol, benzoic acide, and water at a volumetric ratio of (25:7:3:1:1:1:62). A mixture of sucrose and Brewer's yeast (4:1) was used as adult food.

Insecticidal bioassay

A preliminary screening of *C. capitata* was performed using a single-dose test according to Molina et al. (2010) with some modifications. Thus, the first 10 instar larvae were transferred to plastic recipients (30 × 70 mm) containing 5 g of diet (see insect rearing) mixed with 0.5 g of freeze-dried fermentation of selected actinobacterial isolates. The control receives 0.5 g of freeze-dried Bennett's liquid medium. Five repetitions were conducted for each experiment and the pupation was recorded after 14 days of incubation.

Chemical characterization

Chemical reagents

Fast Blue B salt, physostigmine, and 1-naphthyl acetate were purchased from Sigma (Germany). The palladium chloride (was obtained from Riedel-de Haën, the methyl yellow from Acros organics and the molybdatophosphoric acid from Fluka. Silica gel G/UV254 plates were purchased from Macherey-Nagel & Co., with the thickness between 0.2 and 0.25 mm. Other reagents were of analytical grade.

Detection of chemical insecticide families by TLC

The detection of chemical insecticide families was conducted for the most promising isolates from the biological screening on thin layer chromatography (TLC) using specific reagents of each chemical insecticide family: (i) for the organophosphates (OP), the crude extract was applied onto the TLC plate and chromatographed in a mobile phase consisting of n-hexane-acetone (8:2 v/v). The plates were first placed under UV radiation at the wavelength of 254 nm and sprayed with a 0.5% solution of palladium (II) chloride in 10% HCl then heated (Koujiro et al., 1997), an active yellow-brown to black colored spots indicating a possible production of an insecticide of organophosphate family. (ii) for the carbamates (CA), developing solvent was benzene-ethyl methyl acetone (19:1 v/v). The orange or purple specific spots were visualized after migration by treating the chromatogram with an ethanolic solution of Fast Blue B followed by 20% of aqueous solution of NaOH and viewed under short UV light (254 nm) (Tewari and Singh, 1979); (iii) for the pyrethroids (PY) family, the mobile phase consisted of n-hexane-benzene-acetone (9:1:1 v/v) then after, plates were sprayed with molybdatophosphoric acid and heated to 120°C to visualize gray spots in a yellow background (Ogierman and Silowiecki, 1981); (iv) for the detection of organochlorines (OC), the hexane-chloroforme-acetone (9:3:1 v/v) was used as developing solvent. The TLC plates were sprayed with the N,N-Dimethyl-p-phenylazoaniline (methyl yellow), and red spots were viewed under UV radiation at the wavelength of 254 nm (Krzeminski and Landmann, 1963). For all tests, the colored spot positions were determined using the retardation factor (Rf).

Screening for acetylcholinesterase inhibitors

In order to determine whether selected isolates have an insecticidal activity on insect's nervous system a screening assay was conducted for acetylcholinesterase (AChE) inhibition according to the method of Zhongduo et al. (2009). Thus, 500 U of acetylcholinesterase (EC3.1.1.7, Sigma product No. C2888) was first dissolved in 500 mL Tris-hydrochloric acid buffer (0.05 mol L^{-1}, pH = 7.8) containing 500 mg BSA (Sigma, Germany) and 100 µg of crude extract of actinobacteria isolate was dissolved in 1 mL methanol and applied to silica gel TLC plate. The plate was dried and sprayed with enzyme and 1-naphthyl acetate solution (150 mg of 1-naphthyl acetate dissolved in 40 mL of ethanol and diluted with 60 mL of distilled water). Then, it was put in a closed humid environment and kept at 37°C for 20 min. Subsequently, the Fast Blue B salt solution (50 mg dissolved in 100 mL of distilled water) was sprayed onto TLC plate and the inhibition of the enzyme-substrate reaction by the produced insecticide caused the formation of an azo dye resulting in bright zones on a purple colored background (Sherma and Fried, 2003). Physostigmine, known as acetylcholinesterase inhibitor, was used as positive control and the methanol as negative control.

Statistical analyses

All experiments were conducted in a randomized design and the data obtained were compared by one way ANOVA, and means were compared by Tukey's multiple range test, with the level of significance set at $p<0.05$. The lethal concentration (LC_{50}) values were calculated using the EPA computer probit analysis program (Version 1.5). The lethal time (TL_{50}) were calculated according to the SAS LIFEREG procedure fitting a Weibull model to the time interval data with single censored-time observation using JMP version 8.0.

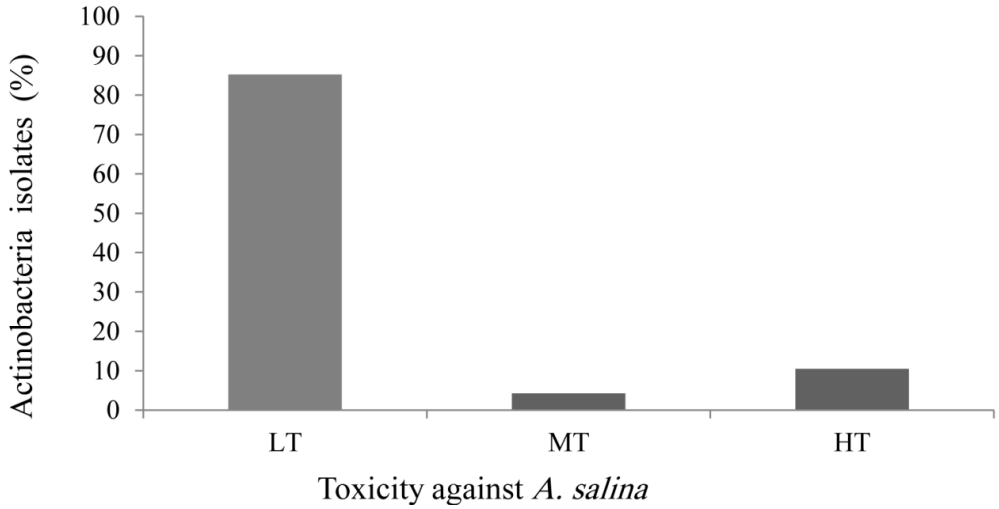

Figure 1. Toxicity of Moroccan actinobacterial isolates against *A. salina*. LT, low toxic isolate with a corrected mortality less than 50%; MT, moderate toxic isolate with a corrected mortality ranged from 50 to 80 %; HT, highly toxic isolate with a corrected mortality ranged from 80 to 100%.

Table 1. Lethal concentration LC_{50} (µg mL^{-1}) and time mortality TL_{50} (h) of the most active isolates using brine shrimp bioassay.

Isolate	LC_{50} (µg mL^{-1})	95% confidence limits		TL_{50} (h)
		Lower	Upper	
OS46	0.260	0.257	0.278	<1 h
37	0.342	0.293	0.445	5.20 ± 0.02
B62	0.840	0.811	0.932	<1 h
B56	1.094	1.043	1.256	2.29 ± 0.04
PH33	1.352	1.343	1.394	<1 h
AS1	2.604	2.550	2.799	3.59 ± 0.04
AS2	2.862	2.834	3.035	2.29 ± 0.04
B89	5.285	4.949	6.130	<1 h
CB33	7.168	6.859	8.131	6.66 ± 0.03
D51	10.893	10.429	12.358	7.94 ± 0.03
OS5	11.265	10.641	12,354	22.79 ± 0.11
B42	23.775	21.154	26.986	11.21 ± 0.06

RESULTS

Determination of anti-brine shrimp activity

The primary biological screening results show that out of 210 isolates, 22 (10.48%) were highly toxic against brine shrimp with a corrected mortality that ranged from 80 to 100%, 9 isolates (4.29%) were moderately toxic with a corrected mortality ranged from 50 to 80% while, 179 isolates (85.24%) have a corrected mortality less than 50% (Figure 1). Among the 22 highly active actinobacteria isolates, only 12 showed 100% brine shrimp mortality at the concentration of 100 µg mL^{-1}, and their corresponding lethal concentration (LC_{50}) and time mortality (TL_{50}) are given in Table 1. Thus, the 12 selected actinobacteria can be divided into two groups: (i) the highly toxic rapid group (HTR) including isolates which caused 50% of *A. salina* mortality in less than 1 h. Among these and except isolate B89 with a LC_{50} of 5.285 µg mL^{-1}, the others (OS46, B62 and PH33) were the most toxic with a LC_{50} of 0.260, 0.840 and 1.352 µg mL^{-1} respectively ; (ii) the highly toxic slow group (HTS) includes isolates 37, B56, AS1, AS2, 0S5, B42, D51 and CB33 which caused 50% of *Artemia salina* mortality in more than 1 h. Among this group, the isolates 37 and B56 were the most toxic with a respectively LC_{50} of

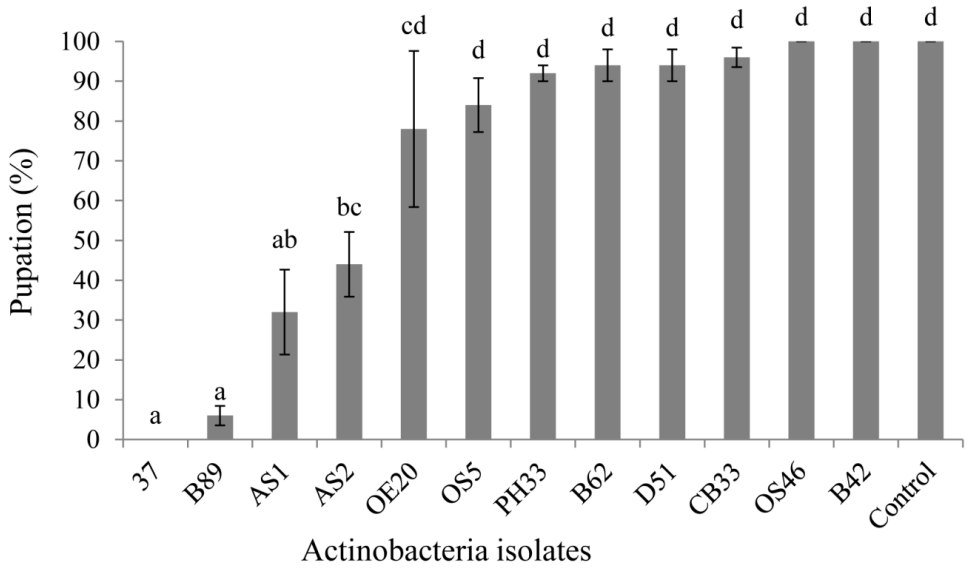

Figure 2. Insecticidal activity of the most active actinobacterial isolates on larval pupation of *Ceratitis capitata* after 7 days of exposure (means followed by different letters with in a column are significantly different; Tukeys HSD, $p < 0.05$).

Table 2. Chemical screening of the most active isolates on thin layer chromatography; using four specific insecticide reagents.

Isolate	TCL with specific insecticide reagents			
	Pyrethroids	Organophosphates	Organochlorines	Carbamates
37	-	-	R (9.33; 22.67)	-
B89	G (10.53; 22.37; 39.47)	G (12.68) Y (19.72; 32.39; 50.70)	R (30.88)	O (28.38; 56.76)
AS1	-	G (32.05)	-	-
OS5	-	G (25.64)	-	-

Color of reveled spot after staining with specific insecticide reagents are: G, grey; Y, yellow; R, red; O, orange; P, purple; -: no active spot; numbers in brackets represent the Rfx100.

0.342 and 1.094 µg mL^{-1}.

Insecticidal activity against *C. capitata*

The biological control against the first-instar larvae of *C. capitata* (Figure 2) showed that the percentage of pupation ranged from 0 to 100%, and only isolates 37, B89, AS1 and AS2 caused a significant reduction in larval pupation compared to control as determined by Tukey's Student range test ($p < 0.05$). No pupation was obtained from treated larvae with isolate 37. On the other hand, the remaining isolates showed no significant mortality in comparison to the control with no pupation reduction for isolates B42 and OS46.

Chemical screening

The thin layer chromatography (TLC) of crude extracts showed a diversity of the bioinsecticide compounds produced by the most promising active isolates against the first-instar larvae of *C. capitata* as indicated in Table 2. The variety of colored bands on TLC after staining with palladium (II) chloride, Fast Blue B, molybdatophosphoric acid and methyl yellow showed that the selected actinobacterial isolates gave at least one active spot on TLC plate. Thus, isolate 37 gave a red spot after staining with the methyl yellow indicating that these crude extracts could contain chlorinated insecticide compounds. Furthermore, the isolate AS1 gave a gray spot after spraying with the palladium chloride, a specific reagent for thiophosphate esters and other sulfur compounds. While, isolate B89 exhibits a wide chemical variety with at least one active spot for each specific reagent tested.

Acetylcholinesterase inhibitors

The screening of the most promising isolate as candidate

Table 3. Acetylcholinesterase inhibition bioassay of the crude extracts of the most active isolates at the concentration of 100 µg mL^{-1}.

Isolate	Inhibition of acetylcholinesterase
37	-
B89	+
AS1	+
OS5	+
Physostigmine	+
Methanol	-

+, Active spot; -, no active spot.

for the production of acetylcholinesterase inhibitors indicate that when the crude extract of the isolates AS1, OS5 and B89 was used in the TLC-bioautographic assay at the concentration of 100 µg mL^{-1}, a bright zones on a purple colored background were clearly observed. Conversely, both the isolates 37 and physostigmine failed to give an active spots on the TLC plates (Table 3).

DISCUSSION

Trend in insect pest control have been shifted from the reliance in conventional chemical pesticides to natural product such as microbial insecticide. The discovery of new active metabolites must be followed by adequate biological testing (Naine et al., 2012). In this study, we investigated the ability of Moroccan actinobacteria isolates to produce insecticidal compounds against *C. capitata* by combining several screening methods. From the 210 investigated Moroccan isolates, 5.71% showed 100% anti-brine shrimp activity. These isolated could be a potential candidate for the production of insecticidal compounds, and also a source of cytotoxic substances as described by many other authors (Xiong et al., 2004; Anibou et al., 2008; Deng et al., 2008). According to Meyer et al. (1982), LC_{50} value lower than 1000 µg mL^{-1} indicated significant cytotoxic potentials. In our bioassay, LC_{50} value of the twelve most active isolates ranged from 0.26 to 23.77 µg mL^{-1} indicating a high anti-brine shrimp activity. Similar toxicity values were obtained from actinobacteria by many other authors using *A. salina* as a model of screening bioinsecticides compounds from actinobacteria (Tantithanagorngul et al., 2011; Kekuda et al., 2012; Tanvir et al., 2014). However, the TL_{50} values obtained in our study were lower than those obtained by the same authors. The 12 selected actinobacteria isolates were there after tested against the Mediterranean fruit fly *C. capitata* and especially against the first instar which is considered to be, with the second instar, the most crops damaging. In fact, the most food intake is used to expand the cell and body size of first and second instars during the life cycle of this Diptera (Berni et al., 2009).

Our results show that the ingestion of freeze-dried fermentation of the isolates 37, B89, AS1 and AS2 caused a significant pupation reduction (P>0.05) compared to the control after 14 days. Many works have demonstrated that actinobacteria isolates are able to produce bioinsecticides compounds against *C. capitata* (Karthik et al., 2011; Deepika et al., 2012; Saurav et al., 2013). The fact that selected bioactive isolates seem to be differents regrading their morphological characteristics and fermentation (data not shown) suggested that they could produce different active compounds. Thus, a chemical characterization was conducted using thin layer chromatography (TLC) combined with chromogenic reagents; in order to assess the nature and chemical diversity of the produced bioinsecticide metabolites. The chemical screening of the acetic ester extracts by thin layer chromatography (TLC) helped to determine the diversity of the chemical constituents produced by the investigated isolate. The variety of the colored bands and their Rf obtained on TLC plates after staining with specific insecticide reagents represent secondary metabolites diversity in the crude extract and could indicate the mechanism of action of the respective actinobacterial isolates. Indeed, the isolate B89 produce a wide chemical variety of secondary metabolites, while the isolates 37, AS1 and OS5 exhibit less diversity. On the other hand, the production of organophosphate compounds could indicate a mechanism involving the enzyme acetylcholinesterase. Accordingly, only the isolates AS1, OS5 and B89 could act on this enzyme. We also notice that the isolate B89 produced compounds belonging to the four insecticide families investigated and could have therefore more than one mechanism of action. These finding were confirmed by a screening of acetylcholinesterase inhibitors. Indeed, the obtained result showed that except the isolate 37, all selected isolates inhibited the enzyme at the concentration of 100 µg mL^{-1}. In fact the organophosphates acted primarily by phosphorylation of the acetylcholinesterase enzyme at nerve endings (Moss and Henderson, 1999; Walker and Asher, 2005). Consequently, it allowed the accumulation of acetylcholine at parasympathetic neuroeffector junctions and caused insect poisoning (Waxman, 1998). It can be conferred from the study that Moroccan actinobacteria could be an important source of compounds with insecticidal activity. The isolates 37, B89, and AS1 showed a potential for controlling *C. capitata* and could be useful in integrated control against this Pest. The bioassay of selected isolates against the other stages of *C. capitata* and the taxonomic characterization of isolates with important larvicidal activity against the Medfly as well as isolation, purification and structural elucidation of the produced larvicidal bioactive metabolites are under investigation.

Conflict of interests

The authors did not declare any conflict of interest.

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REFERENCES

Abbas N, Ali-Khan HA, Shad SA (2014). Cross-resistance, genetics, and realized heritability of resistance to fipronil in the house fly, *Musca domestica* (Diptera: Muscidae): a potential vector for disease transmission. Parasitol. Res. 113:1343-1352.

Abbott WS (1925). A method of computing the effectiveness of an insecticide. J. Am. Mosq. Control Assoc. 3:302-303.

Aboussaid H, Vidal-Quist JC, Oufdou K, El Messoussi S, Castañera P, González-Cabrera J (2011). Occurrence, characterization and insecticidal activity of *Bacillus thuringiensis* strains isolated from Argan fields in Morocco. Environ. Technol. 32:1383-1391.

Alaoui A, Imoulan A, Alaoui-Talibi ZE, Meziane AE (2010). Genetic structure of Mediterranean fruit fly (*Ceratitis capitata*) populations from Moroccan Endemic Forest of *Argania spinosa*. Int. J. Agric. Biol. 12:291-298.

Anibou M, Chait A, Zyad A, Taourirt M, Ouhdouch Y, Benherref A (2008). Actinomycetes from Moroccan habitats: isolation and screening for cytotoxic activities. World J. Microb. Biotechnol. 24:2019-2025.

Barakate M, Ouhdouch Y, Oufdou K, Beaulieu C (2002). Characterization of rhizospheric soil streptomycetes from Moroccan habitats and their antimicrobial activities. World J. Microb. Biotechnol. 18:49-54.

Barry JD, Vargas RI, Miller NW, Morse JG (2003). Feeding and foraging of wild and sterile Mediterranean fruit flies (Diptera: Tephritidae) in the presence of spinosad bait. J. Econ. Entomol. 96:1405-1411.

Berdy J (2005). Bioactive microbial metabolites. J. Antibiot. 58:1-26.

Berni J, Rabossi A, Pujol-Lereis L, Tolmasky D, Quesada-Allué L (2009). Phloxine B affects glycogen metabolism in larval stages of *Ceratitis capitata* (Diptera: Tephritidae). Pestic. Biochem. Physiol. 95:12-17.

Bolognesi C (2003). Genotoxicity of pesticides: a review of human biomonitoring studies. Mutat. Res. Rev. Mutat. 543:251-272.

Chueca P, Garcerá C, Urbaneja A, Molto E (2013). A new mechanised cultural practice to reduce *Ceratitis capitata* Wied. populations in area-wide IPM. Span. J. Agric. Res. 11:1129-1136.

Croft BA (1990). Arthropod biological control agents and pesticides. Wiley-Interscience, New York. p. 723.

De Meyer M, Robertson MP, Peterson AT, Mansell MW (2008). Ecological niches and potential geographical distributions of Mediterranean fruit fly (*Ceratitis capitata*) and Natal fruit fly (*Ceratitis rosa*). J. Biogeogr. 35:270-281.

Deepika T, Kannabiran K, Khanna V, Rajakumar G, Jayaseelan C, Santhoshkumar T, Rahuman A (2012). Isolation and characterisation of acaricidal and larvicidal novel compound (2s,5r,6r)-2-hydroxy-3,5,6-trimethyloctan-4-one from *streptomyces* sp. against blood-sucking parasites. Parasitol. Res. 111:1151-1163.

Deng Y, Hu J, Liu L, Wang N, Wang S (2008). Screening for marine actinomycetes producing insecticidal metabolites. J. Biotechnol. 136: S533.

Dominiak BC, Ekman JH (2013). The rise and demise of control options for fruit fly in Australia. Crop Prot. 51:57-67.

Ferencz L, Balog A (2010). A pesticide survey in soil, water and foodstuffs from central Romania. Carpath. J. Earth. Environ. 5:111-118.

Herbert AK (2010). The spinosyn family of insecticides: realizing the potential of natural products research. J. Antibiot. 63:101-111.

Hsu J, Feng H (2006). Development of resistance to spinosad in oriental fruit fly (Diptera: Tephritidae) in laboratory selection and cross-resistance. J. Appl. Entomol. 99:931-936.

Jemâa JMB, Bachrouch O, Allimi E, Dhouibi M (2010). Field evaluation of Mediterranean fruit fly mass trapping with Tripack® as alternative to malathion bait-spraying in citrus orchards. Span. J. Agric. Res. 8:400-408.

Karthik L, Gaurav K, Rao K, Rajakumar G, Rahuman A (2011). Larvicidal, repellent, and ovicidal activity of marine actinobacteria extracts against *culex tritaeniorhynchus* and *culex gelidus*. Parasitol. Res. 108:1447-1455.

Kekuda PTR, Shobha KS, Onkarappa R, Goutham SA, Raghavendra HL (2012). Screening biological activities of a *Streptomyces* species isolated from soil of Agumbe, Karnataka, India. Int. J. Drug. Dev. Res. 4:104-114.

Koujiro F, Chie N, Yasufumi K, Hideki S, Ryozo O (1997). Application of high-performance thin-layer chromatography for the detection of organophosphorus insecticides in human serum after acute poisoning. J. Chromatogr. B. 704: 369-373.

Krzeminski LF, Landmann WA (1963). Methyl yellow as a spray reagent in the paper chromatography of chlorinated hydrocarbon pesticides. J. Chromatogr. B. 10: 515-516.

Kumari B, Madan V, Kathpal T (2008). Status of insecticide contamination of soil and water in Haryana, India. Environ. Monit. Assess. 136:239-244.

Lance DR, Woods WM, Stefan M (2014). Invasive insects in plant biosecurity: Case study - Mediterranean fruit fly. In: Gordh G, McKirdy S (Eds.), The Handbook of plant biosecurity. Springer, Netherlands. pp. 447-484.

Liou Q (2000). The research of new method of biomonitoring. Environmental Monitoring in China 16:38-40.

Liu H, Qin S, Wang Y, Li W, Zhang J (2008). Insecticidal action of Quinomycin A from a *Streptomyces* sp. KN-0647, isolated from a forest soil. World J. Microb. Biotechnol. 24:2243-2248.

Malacrida AR, Gomulski LM, Bonizzoni M, Bertin S, Gasperi G, Guglielmino CR (2007). Globalization and fruitfly invasion and expansion: the medfly paradigm. Genetica 131:1-9.

Meyer BN, Ferrigni NR, Putnam JE, Jacobsen LB, Nichols DE, McLaughlin JL (1982). Brine shrimp: a convenient general bioassay for active plant constituents. Planta Med. 45: 31-34.

Molina CA, Cana-Roca JF, Osuna A, Vilchez S (2010). Selection of a *Bacillus pumilus* strain highly active against *Ceratitis capitata* (Wiedemann) larvae. Appl. Environ. Microbiol. 76: 1320-1327.

Moss DW, Henderson AB (1999). Clinical enzymology. In: Burtis CA, Ashwood ER, Tietz NW (Eds.), Tietz Textbook of Clinical Chemistry. Saunders, Philadelphia. pp. 617-621.

Naine JS, Nasimunislam N, Vaishnavi B, Mohanasrinivasan V, Devi SC (2012). Isolation of soil Actinomycetes inhabiting Amrithi forest for the potential source of bioactive compounds. Asian J. Pharm. Clin. Res. 5: 189-192.

Oerke EC (2005). Crop losses to pests. J. Agric. Sci. 144:31.

Oerke EC, Dehne HW (2004). Safeguarding production - losses in major crops and the role of crop protection. Crop Prot. 23: 275-285.

Ogierman L, Silowiecki A (1981). Thin-layer and gas chromatographic separation of the stereoisomers of some synthetic pyrethroids. Chromatographia 14:459-461.

Omura S (2011). Microbial metabolites: 45 years of wandering, wondering and discovering. Tetrahedron 67: 6420-6459.

Osman KA (2011). Pesticides and human health. In: Stoytcheva M (Ed) Pesticides in the modern world - Effects of pesticides exposure. InTech, Rijeka. pp. 205-230.

Popp J, Peto K, Nagy J (2013). Pesticide productivity and food security - A review. Agron. Sustain. Dev. 33:243-255.

Preetha G, Stanley J, Suresh S, Samiyappan R (2010). Risk assessment of insecticides used in rice on miridbug, *Cyrtorhinus lividipennis* Reuter, the important predator of brown planthopper, *Nilaparvata lugens* (Stal.). Chemosphere 80:498-503.

Ravensberg WJ (2015). Commercialisation of Microbes: Present Situation and Future Prospects. In: Lugtenberg B (Ed) Principles of Plant-Microbe Interactions. Springer International Publishing. pp. 309-317.

Rivero A, Vézilier J, Weill M, Read AF, Gandon S (2010). Insecticide control of vector-borne diseases: When is insecticide resistance a problem. PLoS Pathog. 6: 1-9.

Ruberson JR, Nemoto H, Hirose Y (1998). Pesticides and conservation of natural enemies in pest management. In: Barbosa P (Ed) Conservation

Biological Control. Academic Press, San Diego CA. pp. 207-220.
Saurav K, Rajakumar G, Kannabiran K, Rahuman A, Velayutham K, Elango G, Kamaraj C, Zahir A (2013). Larvicidal activity of isolated compound 5-(2,4-dimethylbenzyl) pyrrolidin-2-one from marine *streptomyces* VITSVK5 sp. against *rhipicephalus (boophilus) microplus, anopheles stephensi*, and *culex tritaeniorhynchus*. Parasitol. Res. 112:215-226.
Sherma J, Fried B (2003). Handbook of thin layer chromatography. Marcel Dekker, New York-Basel. p. 703.
Shiomi K, Hatae K, Hatano H, Matsumoto A, Takahashi Y, Jiang CL, Tomoda H, Kobayashi S, Tanaka H, Ōmura S (2005). A new antibiotic, antimycin a9, produced by *streptomyces* sp. K01-0031. J. Antibiot. 58:74-78.
Stark JD, Vargas RI, Miller N (2004). Toxicity of sprinosad in protein bait to three economically important tephritid fruit fly species (Diptera: Tephritidae) and their parasitoids (Hymenoptera: Braconidae). J. Econ. Entomol. 97:911-915.
Su T, Cheng ML (2013). Laboratory selection of resistance to Spinosad in *Culex quinquefasciatus* (Diptera: Culicidae). J. Med. Entomol. 51:297-488.
Tantithanagorngul W, Sujitwanit A, Piluk J, Tolieng V, Amorn P, Sangvanich P, Palaga T, Puthong S, Thamchaipenet A, Pinphanichakarn P (2011). Screening for brine shrimp larvicidal activity of *Streptomyces* isolated from soil and anti-tumor activity of the active isolates. Aust. J. Basic Appl. Sci. 5: 15-22.
Tanvir R, Sajid I, Hasnain S (2014). Biotechnological potential of endophytic actinomycetes associated with Asteraceae plants: isolation, biodiversity and bioactivities. Biotechnol. Lett. 36: 767-773.
Tewari SN, Singh R (1979). Identification and detection of carbamate pesticides in autopsy tissues using thin-layer chromatography. Fresenius. J. Anal. Chem. 294:287-287.
Vargas RI, Miller NW, Stark JD (2003). Field trials of spinosad as a replacement for Naled, DDVP, and malathion in methyl eugenol and cue-lure bucket traps to attract and kill male oriental fruit flies and melon flies (Diptera: Tephritidae) in Hawaii. J. Econ. Entomol. 96:1780-1785.
Walker JP, Asher SA (2005). Acetylcholinesterase-based organophosphate nerve agent sensing photonic crystal. Anal. Chem. 77:1596-1600.
Waxman MF (1998). The agrochemical and pesticides safety handbook. CRC Press, New Yok. p. 640.
Xiong L, Li J, Kong F (2004). *Streptomyces* sp. 173, an insecticidal micro-organism from marine. Lett. Appl. Microbiol. 38:32-37.
Zhiyu H, Sanzhen L, Hao H, Yaojian H, Wenjin S (2000). A rapid screening assay for insecticidal antibiotic produced by marine actinomycetes. Bull. Mar. Sci. 19:36-41.
Zhongduo Y, Xu Z, Dongzhu D, Zhuwen S, Mingjun Y, Shuo L (2009). Modified TLC bioautographic method for screening acetylcholinesterase inhibitors from plant extracts. J. Sep. Sci. 32:3257-3259.