المملكة العربية السعودية
الخطة الوطنية للعلوم والتقنية والابتكار

Full Research Proposal
Application Form

PLAN	PROGRAM	CODE / NUMBER
The First Five-Year STI Plan	ADVANCED AND STRATEGIC TECHNOLOGIES	ASTP-10
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	TRACK	
	Environmental Health	
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	Environmental Health	
Proposal Title English	**Native mosquito larvicidal bacteria isolates as new candidates for control of mosquito-borne diseases in Saudi Arabia**	
Proposal Title Arabic	**ترشيح عزلات بكتيرية محلية جديدة قاتلة ليرقات البعوض في المعركة ضد الأمراض المنقولة بالبعوض في المملكة العربية السعودية**	
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PROJECT INFORMATION

Project Title	**Native mosquito larvicidal bacteria as new candidates for control of mosquito-borne diseases in Saudi Arabia**				
Technology Area	**Medical and Health**				
Track	**Environmental Health**				
Sub-Track	**Environmental Health**				
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Proposed Starting Date	**One month from acceptance**				
Research Team	**Senior Personnel**				
	No.	**Name**	**Research Status**	**Role**	**Area of Specialization**
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	Other Personnel				
	6	Two persons		Assistant researcher	
	7	One person		Ph.D. Student	
	8	One person		M.Sc. Student	
	9	Two persons		Technicians	
	10	-		Project Manager	
	11	One person		Other (assistant in field collections)	
	Consultants				
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Is this Proposal being submitted to any other funding institution ?	☐ Yes, Specify			1. 2. 3.	
	☑ No				

TABLE OF CONTENTS

SUMMARY (English)

Control of insect vectors of diseases plays a vital role in the fight to improve human health around the world. In the Kingdom of Saudi Arabia (KSA), there are a number of different types of mosquitoes transmitting different types of life-threatening diseases within the Saudi community. Malaria, transmitted by *Anopheles arabiensis*, is prevalent in the southern regions; dengue fever, transmitted by *Aedes aegypti*, is present in the western regions; and Rift Valley Fever, transmitted by *Aedes caspius* and other *Culex* species, is found in various regions of the Kingdom. The Saudi Government is currently supporting considerable efforts to prevent mosquito-borne diseases, especially during Hajj (pilgrimage) time, by controlling these vectors. The goal is to keep the number of insect vectors below the level of economic damage. However, there is an urgent need for effective biocontrol methods that will eliminate the vectors but at the same time maintain a clean and safe environment. Current practices of using broad-spectrum chemical insecticides not only lead to mosquito resistance (Al-Sarar, 2010) but also leave both soil and groundwater contaminated with hazardous chemicals, which may further complicate the situation by causing other human diseases.

The current research project seeks to regain the balance of nature by developing and then implementing biocontrol measures against insect vectors, while keeping the environment safe and unpolluted by chemicals insecticides. Thus, in line with the Saudi governmental plans, we propose this project to investigate environmentally safe biocontrol strategies for controlling mosquito vectors in KSA. The project will target the widely distributed *Culex* and *Aedes* mosquito vectors, using new native strains of environmentally safe bacteria. Certain native bacterial isolates from *Bacillus thurengiensis* and the nematodal symbiont bacteria, *Photorhabdus luminescens* & *Xenorhabdus nematophilus*, have previously shown remarkable larvicidal activity in preliminary studies in our labs. The aim of this research is to study several native bacterial isolates in detail, from bacteriological, molecular, biochemical and toxicological points of view. We believe that this study will lead to the development of new native larvicidal bacteria as suitable environmentally safe candidates in the battle against mosquito vectors in KSA, and thereby will limit the necessity for chemical insecticides, overcome the problem of insecticide resistance by mosquito vectors, and minimize fatal mosquito-borne diseases within the Saudi community. A patenting of commercial products for mosquito biocontrol could be achieved. Moreover, one important target of this 2-years project is to develop and retain a national manpower and expertise in medical and health sciences research and establish a laboratory dedicated to the development of future long-term work on biocontrol of insect vector in KSA.

SUMMARY (Arabic):

لقد كانت و ما زالت الحشرات الناقلة للأمراض تشكل واحدةً من أهم المشاكل المَرَضية للإنسان في العصر الحديث. ففي المملكة العربية السعودية ينتشر العديد من أنواع البعوض الناقل للعديد من الأمراض المهددة لحياة الإنسان في المجتمع السعودي، مثل، مرض الملاريا الذي تنقله بعوضة الأنوفيليس العربية في المناطق الجنوبية، وحمى تهشم العظام (حمى الدنج) الذي تنقله بعوضة الأيديس المصرية في المناطق الغربية، ومرض حمى الوادي المتصدع الذي تنقله بعوضة أيديس كاسبياس والكيوليكس المنزلية في مناطق أخرى مختلفة من المملكة. ولهذا، فقد أولت الحكومة السعودية اهتماما بالغا بدعم مشاريع بحثية كبيرة ومتعددة لمقاومة تلك الحشرات الضارة لمنع انتشار تلك الأمراض المنقولة بالبعوض وخاصة في موسم الحج. فعلى الرغم من أهمية العمل على إبقاء أعداد هذه الحشرات تحت خط الإضرار باقتصاد الوطن وصحة الإنسان، إلا أن هناك احتياجا ملحا للمحافظة على نظافة البيئة في نفس الوقت، مما دفع المختصين بالتركيز على استخدام مبيدات حياتية آمنة وغير ملوثة للبيئة. فلطالما أدى استخدام الإنسان لمدى واسع من المبيدات الكيميائية إلى ظهور ليس فقط ممانعة للبعوض ضدها (مثلا Al-Sarar, 2010) ولكن أيضا تلوث كلا من التربة والمياه الجوفية بملوثات كيميائية والتي ، من ناحية أخرى، قد فاقمت من الوضع حيث أدت إلى ظهور أمراض أخرى تهدد صحة الإنسان.

يعد هذا المشروع البحثي نداءا هاما لاستعادة التوازن الطبيعي من خلال تطبيق برامج مكافحة حياتية ضد نواقل الأمراض، وفي نفس الوقت، تكون آمنه للبيئة. واستجابةً لمبادرة الحكومة السعودية بتشجيع البحث في هذا الصدد، نقدم هذا المقترح البحثي دعما لإستراتيجية المكافحة الحياتية الآمنة للبيئة ضد البعوض الناقل للأمراض في المملكة العربية السعودية. وعليه، فإن الدراسة الحالية تستهدف نوعين من أكثر أنواع البعوض انتشارا في المملكة وهما من جنسي الكيوليكس والأيديس، وذلك باستخدام عزلات محلية من البكتريا الآمنة للبيئة والمتخصصة فى إصابة الحشرات دون التأثير على الإنسان وحيوان المزرعة. فلقد أظهرت نتائج دراسة مبدئية في مختبراتنا أن بعض العزلات المحلية من بكتريا الباسيلاس ثورينجيينسيس (*Bt*) وبكتريا النيماتودا التكافلية، فوتورابداس ليومينيسينس (*Pl*) و زينورابداس نيماتوفيلاس (*Xn*) أظهرت سمية عالية ضد بعض أنواع البعوض. ومن ثم، فقد تم اقتراح هذا المشروع البحثي لدراسة تلك العزلات البكتيرية المحلية (وغيرها) تفصيليا من الناحية الميكروبية والجزيئية والكيموحياتية والسمية. وعليه، فنحن نأمل بأن تنتهي هذه الدراسة إلى تعزيز وترشيح عزلات بكترية محلية، قاتلة للبعوض وآمنة للبيئة، كسلاح أساسي في المعركة ضد البعوض الناقل للأمراض في المملكة العربية السعودية مما يؤدي إلى الحد من استخدام المبيدات الكيميائية الملوثة للبيئة، والتغلب على مشكلة مقاومة البعوض للمبيدات المستخدمة لمقاومتها حاليا، ومن ثم، الحد من الأمراض المنقولة بالبعوض في المجتمع السعودي. ومن المتوقع أن يؤدي هذا المشروع إلى تسجيل اكتشاف بكتريا محلية جديدة مقاومة للبعوض. كما نأما أن يتم تأهيل فريق بحثي في مجال أبحاث الطب والصحة العامة من خلال مقاومة ناقلات الأمراض، والتأسيس لمختبر يضمن استمرارا طويل المدي في مجال أبحاث المقاومة الحياتية في المملكة العربية السعودية.

1. INTRODUCTION

Millions of Muslims perform a religious pilgrimage, the "Hajj", to Mecca in the Kingdom of Saudi Arabia (KSA) every year. During the Hajj, pilgrims face numerous health hazards since the gathering of such a large number of people during this time brings the attendant health risks of contracting infectious diseases (reviewed by Ahmed *et al.,* 2006). This religious gathering, in fact, highlights some of the world's most important public health and disease control problems. The potential spread of infectious disease and other health risks during this time pose a very challenging problem for Saudi public health specialists. Furthermore, the possibility of emerging infectious diseases turning into potential epidemics is of real concern. Prior to each Hajj season, the Saudi authorities are continually refining and improving their disease prevention measures for the management of Hajj.

There are a number of different species of mosquitoes transmitting different types of life-threatening mosquito-borne diseases in KSA. Rift Valley fever, which is transmitted by *Aedes caspius*, is prevalent in southern and eastern regions in KSA (Ahmad, 2000), where there were 516 persons with suspected severe disease recorded in 2000. Dengue fever, which is transmitted by *Aedes aegypti* (Charrel, *et al.,* 2001 and Madani, 2005), was recorded in both Mecca and in Jeddah in 2001. Globally, an estimated 300–500 million clinical cases of malaria, transmitted by the genus *Anopheles* (Harbach, 1994), occur annually, with up to 3 million deaths, most of them in children under five years old. In KSA, malaria is confined mainly in the southern region, where it is transmitted by *Anopheles arabiensis* (El-Refaie *et al.,* 1984; El-Sebai *et al.,* 1987; Abdullah and Merdan, 1995 and Malik *et al.,* 1998; Abdoon and Alshahrani, 2003). KSA is thus affected by a number of life-threatening mosquito-borne diseases, and the Saudi Government is therefore supporting strenuous efforts to control such diseases by controlling the mosquito vectors.

The Saudi Ministry of Health has currently developed and implemented effective plans to minimize the threat of mosquito-borne diseases especially during Hajj time. These measures are aimed at keeping the number of insect vectors to a minimum and certainly below the level of economic damage. Nevertheless, the urgent need for a clean and safe environment has forced the majority of scientists to move away from chemical control of insect vectors to focus on the utilization of environmentally safe biocontrol agents. Unfortunately, the use of broad-spectrum chemical insecticides has not only led to mosquito resistance (Al-Sarar, 2010) but also to soil and groundwater contamination with hazardous chemicals. Other methods are urgently needed to keep insect vectors below the level where they pose a threat to human health but at the same time to keep the environment safe and non-hazardous to humans. Implementing alternative biocontrol agents to reduce vector transmission of disease is thus the simplest and most effective way forward. We believe that periodical introduction of native strains of bacterial bioinsecticides will add new weapons to the armory for managing mosquito-borne diseases, which will also prevent mosquito resistance. So, the use of a new alternative approach to biological control is urgently needed to open a new front in the fight against the threat to human health posed by mosquito-borne diseases in KSA. To the best of our knowledge, biological control of mosquito vectors in KSA has so far not been widely used.

The present study is designed to establish a reduction in the disease-carrying capacity of two widely distributed mosquito species in different regions of KSA, *Culex pipiens* (Al-Khreji *et al*., 2007) and *Ae. caspius* (Ahmed *et al*., 2011), which are the main vectors of Filariasis and Rift Valley Fever, respectively. Native bacterial isolates from *Bacillus thurengiensis* (*Bt*) and the nematodal symbiont bacteria, *Photorhabdus luminescens* (*Pl*)

and/or *Xenorhabdus nematophilus* (*Xn*), will be tested as mosquito larvicides. Our preliminary tests using toxins of these bacteria and one isolated native *Bacillus thurengiensis* have shown promising toxicity against the larvae of some mosquito species. The aim of this research project is to extend this research towards developing new methods for overcoming mosquito resistance to insecticides and to participate in solving the serious public health problem of mosquito-borne diseases in KSA, and may be in other mosquito endemic countries, using safe and effective native mosquito larvicidal bacteria. During the course of this 2-year research project, we will establish local colonies of mosquito vectors and larvicidal bacterial strains, a laboratory dedicated to cutting-edge technology in the field of biological control which could help in training young scientists in the skills required to develop the field of biological control in KSA. Finally, we believe that local mosquitocidal bacterial isolates will be more suitable for the Saudi environmental weather (for the formulated pure toxins as well as for the spore for its temporarily period of activity) as previously suggested by the WHO.

2. PROJECT OBJECTIVES:

The objectives underlying the research described here are to investigate and develop mosquito vector control strategies that rely on the effectiveness of native bacterial isolates as mosquito biolarvicidal agents. These bacteria/bacterial products will be tested against larvae of a known insecticide-resistant filarial vector *Cx. pipiens* (Al-Sarar, 2010) and the Rift Valley vector *Ae. caspius* in KSA (Balkhy and Memish, 2003). The work will be carried out with special references to the well-known mosquitocidal bacterium, *B. thurengiensis* subspecies *israelensis*. Achieving these objectives is summarized as follows:

1- Rearing experimental mosquitoes.

2- Raising native larvicidal *B. thurengiensis* bacterial isolate(s).

3- Raising entomopathogenic nematodes and their bacterial symbiont(s).

4- Conducting larvae toxicity bioassays (LC_{50} and LC_{90}).

5- Testing the histopathological effects of the larvicidal native bacterial isolates on larval midgut.

3. LITERATURE REVIEW

Insects spread illness as disease vectors. They either produce potentially lethal toxins that are associated with allergic reactions or even death, or they transmit disease-causing organisms. Methods for preventing insect-borne illness include avoidance, vector control programs and insect repellents. Mosquitoes of the genera *Aedes*, *Culex* and *Anopheles* are members of the order Diptera, suborder Nematocera, in the family Culicidae. They are found in tropical and subtropical zones throughout the world, and are responsible for the transmission of a number of viral and parasitic human pathogens (Tolle, 2008). Mosquitoes are the most important arthropod vector of parasitic diseases worldwide. They have a cosmotropical distribution between 30°N and 20°S (Christophers, 1960; Knight and Stone, 1977), and prefer human habitats, using small water sources such as tires, plant pots and water storage containers for oviposition (Tabachnick, 1991). The life cycle for most mosquito species is dependent on a blood meal to start ovulation, and eggs are laid individually or in groups on water surfaces. Eggs hatch thereafter into larvae in water and rapidly develop to pupae then to the adult stage within days through a complete metamorphosis.

It has been estimated that two-thirds of the world's population are affected by mosquito-borne diseases, and several million die from these diseases annually (Tolle, 2008). The most important genera involved with human disease pathogens are *Anopheles* (for malaria and Filariasis), *Aedes* (for yellow fever, dengue fever Rift Valley fever), and *Culex* (West Nile, Japanese encephalitis, Filariasis and Rift Valley fever). It is, in fact, very costly to combat these diseases, to the extent that it can cripple the economic growth of countries where the diseases are endemic. Control strategies to reduce vector populations using mainly insecticides, and drugs to kill the causative agents, have developed over the decades. However, both vectors and parasites of some mosquito-borne diseases have developed resistance against many widely used pesticides and drugs, respectively. Although vaccines would be an effective option for vector-borne diseases, they have only been developed against a few pathogens, such as yellow fever and Lyme disease. Thus, there is an urgent need to create alternative new strategies for controlling vector-borne diseases.

In the KSA, there are many species of mosquito vector transmitting different types of life-threatening diseases (Madani, 2005; Al Arishi *et al.*, 2001; Abdoon, 2004; Abdoon and Alsharani, 2003; Abdoon, and Ibrahim, 2005; Abdullah, and Merdan, 1995; Ahmad, 2000; Al-Hazmi, *et al.*, 2003; Al-Hazmi *et al.*, 2005; Al-Khreji *et al.*, 2007, Alahmed *et al*. 2007, Al-Ghamdi *et al.*, 2008, Alahmed *et al.*, 2009 and Ahmed *et al.*, 2011). The following are briefed examples of mosquito-borne diseases transmitted by different types of mosquitoes in KSA.

Dengue Fever

In the western regions of KSA, dengue fever was originally isolated in Jeddah in 1995 from six patients (Zaki 1997; Madani 2005, Al-Hazmi, *et al.*, 2005) and is the most prevalent and dangerous mosquito-borne viral disease of humans in this important region. It is transmitted by the mosquito vector, *Ae. aegypti*, and caused by four dengue virus serotypes (DEN-1, DEN-2, DEN-3 and DEN-4) of the genus *Flavivirus*. Scientists believe that these viruses are transmitted from sheep or goats to humans by the mosquito bites of *Ae. aegypti* (Madani 2005) or by direct contact with these animals. In KSA, these viruses were referred to as Al-Khumra (a district in Jeddah) virus (ALKV) (Charrel *et al.*, 2001). ALKV was detected in other important regions in KSA like Makkah (Khana, *et al.*, 2008) and Najran (Memisha *et al.*, 2010 and Madani *et al.*, 2011). This disease constitutes a real threat to not only Pilgrims but also to the whole Saudi community. The following table shows Dengue fever cases in different major cities from 2004 to 2009 in KSA [Saudi Ministry of Health, (2009), www.saudigazette.com.sa)].

Regions	Years					
	2004	2005	2006	2007	2008	2009
Riyadh	Not available	Not available	0	1	0	3
Makkah	Not available	Not available	199	182	95	1697
Jeddah	Not available	Not available	1308	243	807	1606
Taif	Not available	Not available	0	0	0	25
Madina	Not available	Not available	7	0	5	2
Jizan	Not available	Not available	29	61	6	15
Najran	Not available	Not available	1	3	0	2
Total	343	406	1544	490	913	3350

Rift Valley Fever

Rift Valley fever (RVF) is another mosquito-borne viral disease that transmitted by *Ae. Caspius* and infects sheep, camels, and goats in different parts of KSA. Humans become infected with the virus either *via* mosquito or *via* direct contact with the infective blood or body fluids of infected animals. RVF was first reported in KSA by Ahmed (2000) as a result of importing infected animals from endemic countries. *Aedes dalzieli*, *Aedes vexans* and *Aedes ochraceus* mosquitoes, which normally feed on the blood of cattle and sheep, are the main vectors known to transmit this disease (Thonnon, *et al.,* 1999). Other mosquito species have been experimentally shown to be capable of RVF transmission, including the *Anopheles* and *Culex* mosquitoes (Diallo, *et al.,* 2000 and Hanafi *et al.,* 2011). Although RVF is not currently a serious disease that KSA is suffering from, the increase in number of mosquito vectors as a result of seasonal rains is of particular concern to specialists (Balkhy and Memish, 2003 and Al-Hazmi, *et al.*, 2003) as it can result in epidemics of RFV.

Filariasis

Filariasis is another mosquito-borne parasitic disease transmitted by *Cx. Pipiens* has been reported in KSA for many years (Sebai *et al.,* 1974(Al-Osaimi *et al.,* 1995) and is now endemic in south-eastern KSA. It has also been reported from the western province and other parts of KSA (Gatus and Khan, 1981 and Omar, 1996). An outbreak of cerebrospinal Filariasis in goats was reported from a farm in Qassim, Central KSA (Mahmoud *et al.*, 2004).

Malaria

Malaria is a major worldwide public health problem in many countries since it causes death mainly among infants and young children (WHO/Regional Office for the Eastern Mediterranean, 2007 and Tolle, 2008). In KSA, malaria is restricted to the southern and south-western regions and some important regions like Medina, Taif, Jeddah and Mecca. *Plasmodium falciparum* is the major endemic species in these areas (Anonymous, 1994 and Al-Arishi *et al.*, 2001). In KSA, malaria is transmitted by *An. Arabiensis* and has been confirmed in different regions (El-Refaie *et al.*, 1984; El-Sebai *et al.*, 1987; Abdullah and Merdan, 1995 and Malik *et al.*, 1998; Abdoon and Alshahrani, 2003, and Al-Omar *et al.,* 2010).

Clearly, KSA is affected with several life-threatening mosquito-borne diseases and the Saudi Government is currently making strenuous efforts to control such diseases and the associated mosquito vectors to prevent the

appearance of this disease, especially in the Hajj time of the year. In fact, the reasons for the emergence of different mosquito-borne diseases in KSA can in part be attributed to the expansion of international travel and trade, especially to Jeddah from everywhere in the world as people gather during Hajj. Jeddah provides perfect breeding conditions for mosquitoes and hence for the transmission of vector-borne disease as it is densely populated, temperate and humid. Moreover, a lack of reliable piped-water supplies means that people in many Saudi communities need to store water in or near their homes, which in turn creates suitable breeding sites for mosquitoes. Moreover, large numbers of disposable containers, tires and discarded food or water vessels provide further breeding sites for mosquitoes.

Use of entomopathogenic bacterial control

For many years, researchers have advocated the use of entomopathogenic microorganisms to control harmful insects. Biocontrol agents such as *B. thurengiensis* and *Bacillus sphaericus* are currently being used to control mosquito vectors (Berry *et al.*, 1987 and Becker, 2000). The idea of using new effective bacterial agents against mosquitoes as alternative to chemical insecticides is an important goal for two main reasons. First, it is unlikely to be subject to cross-resistance, which occurs in insecticide resistance mechanisms (Ffrench-Constant, *et al.*, 2004). Second, the bacterial product will be made from naturally occurring bacteria, so it should be environmentally safe, cheap and effective against the target mosquito vector.

B. thurengiensis (*Bt*) is an aerobic, mesophilic, soil-saprophyte, Gram-positive spore forming bacterium that is closely related to the *Bacillus cereus* group. During sporulation, *Bt* produces distinctive one or more parasporal crystal proteins that are encoded by the *cry* and *cyt* genes, respectively (Logan, 2005). So far, the *cry* toxins had been classified into 59 families (i.e., *cry*-1 to *cry*-59) and two groups of Cyt proteins, based on their amino acid sequence homology (Liang *et al*, 2011). The demonstration of such parasporal crystals is the only characteristic that differentiates between the two taxonomically closely related species, *Bt* and *Bacillus cereus* (Bravo *et al*, 2005). The following table shows specific insecticidal crystal proteins target different insect orders (Schnepf *et al*. 1998).

Target	Type of crystals	References
Lepidopteran larvicidal	Cry 1, Cry 2 and Cry 9	(Crickmore *et al*, 1998; Armengol *et al*, 2007; Gobatto *et al*, 2010)
Coleopteran larvicidal	Cry3, Cry7, Cry8, Cry14, Cry34, Cry35, Cry36, Cry38	Tokcaer, 2003
Dipteran larvicidal	Cry 2, Cry 4, Cry 10, Cry 11, Cry 16, Cry 17, Cry 19, Cry 20, Cry 24, Cry25, Cry 27, Cry 29, Cry 30, Cry 32, Cry 39, Cry 40	(Crickmore *et al*, 1998; Armengol *et al*, 2007; Gobatto *et al*, 2010; Tokcaer, 2003)
Dipteran larvicidal	*Bacillus thurengiensis* subsp. *Israelensis* cytolytic toxins (Cyt)	(Crickmore *et al*, 1998; Armengol *et al*, 2007, Gobatto *et al*, 2010)
Lepidopteran and Dipteran larvicidal	Cry2	Tokcaer, (2003)
Anti-nematode	Cry5, Cry6, Cry12, Cry13, Cry21	Hu *et al*, (2010)
Hymenopteran	Cry22	Tokcaer, (2003)
Anti-cancer	Parasporin (PS)	Abou El-Hag & Safhi (2011)

These delta-endotoxins (located intracellularly) have been in use for a long time as successful bioinsecticides (Schnepf *et al.*, 1998). Schnepf *et al.*, (1998) and Cinar *et al*, (2008) investigated the mode of action of these delta-endotoxins, as shown in Fig. (1). They showed that when susceptible insect larvae ingest *Bt* spore-crystals, the crystal δ-endotoxins are solubilized in the alkaline environment of the midgut and then these protoxins are proteolytically cleaved by midgut proteases into active toxic peptides. The active toxin binds to specific receptors on the surface of midgut cells and is inserted into the membrane to form pores that destroy transmembrane potential, resulting in osmotic lysis of the cells lining the midgut, and fatal consequences to the mosquito (for more details, please see (Brar, *et al.*, 2007).

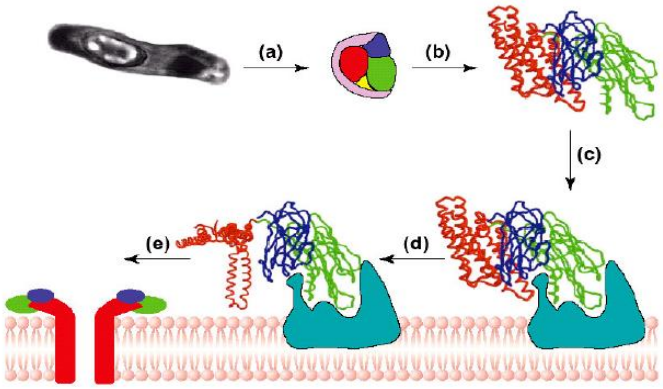


Figure (1) Action Mechanism of Cry delta-endotoxin (de Maagd et al. 2001)

Recent advances in DNA manipulation have led to a worldwide development of commercially applicable *Bt*-based bioinsecticides to specifically control targeted insect orders, including the mosquito. The research interest in *Bt* was initiated in our lab some months ago with the hope of using these products as safe bioinsecticides (Schnepf *et al*. 1998) that would complement and/or replace the chemical-mosquito control program in KSA. This has led us to isolate a number of *Bt* native isolates from different localities across the KSA (Fig. 2). The identity of these native *Bt* isolates was recently confirmed both phenotypically and genotypically using 16S rRNA gene analysis and PCR.

Preliminary Data

The following are some of our preliminary unpublished data showing phase contrast microscopy (Fig. 2 A, B and C), showing crystals and spores of some native *Bt* isolates with different colonial and crystal morphology (Tables 1 and 2), as well as scanning electron microscopy for one native isolate (Fig. 2F). Figure (3) shows a dendrogram of 16 S RNA, illustrating the molecular relatedness of 15 native *Bt* isolates, together with positive controls of the two reference strains *Bt* H14 and BtKD1 as well as two strains of local *B. cereus* and one spore forming local *Bacillus endophyticus*. Of these, one native Saudi *Bt* isolate showed high toxicity against mosquitoes in preliminarily testing, which will be investigated in detail in the current study.

Table 1: Morphological appearance of native 64 Bt isolates.

Colony Type	Characterization	Percentages of *Bt* isolates
A	White, round, flat, and with wavy margin (scalloped-edged)	**85%**
B	White, round, slightly raised center, and with fried egg appearance.	**9%**
C	White, shinny, round, little raised center, and with fried egg appearance with entire margin.	**3%**
D	White, round, mucoid, slightly raised center, and spread out with irregular spike-like margin.	**3%**

Table 2: Diverse morphologies of parasporal crystal presented by Bt isolates.

Group code	Crystal classes	Percentages of isolates
SP	Spherical	56%
G	Irregular	14%
SG	Spherical and irregular	12%
B	Pointed bipyramidal	5 %
BS	Bipyramidal and spherical	3 %
SC	Spherical and cubic	2%
SGR	Spherical, irregular, and rhomboidal	2%
AT	Remain attached to the spore	6%

Figure 2: Micrograph plate shows reference strains *Bt* H14 and BtKD1 (**A** and **B**), and novel local *Bt* isolates with phase contrast micrographs with different crystal shapes (**C**, **D** and **E**), where S: spore and C: crystal; **F**: represents Electron microscopy of *Bt* local isolate (isolate: 6) showing both spherical crystals and spores at ×24,000 magnification.

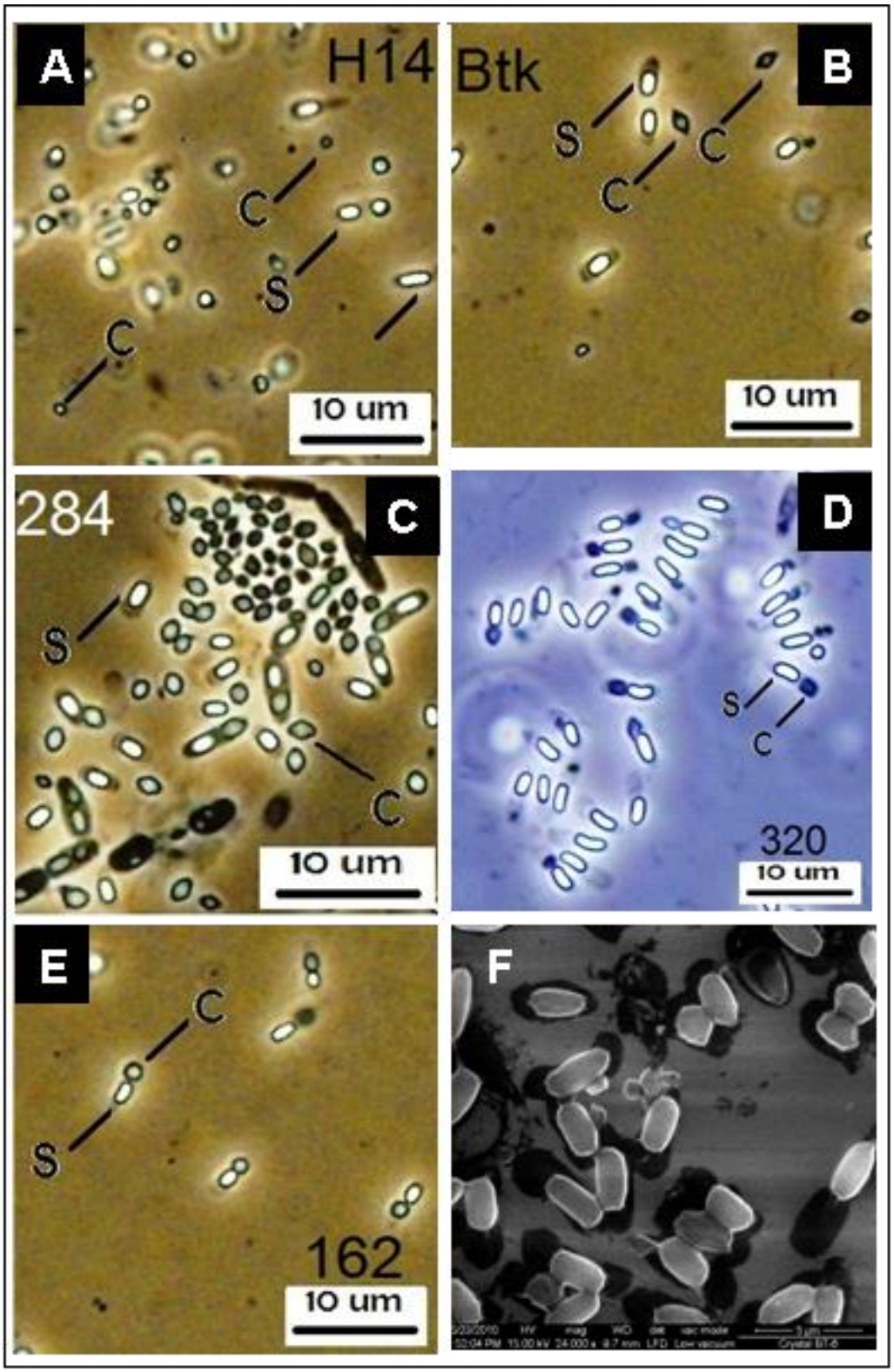
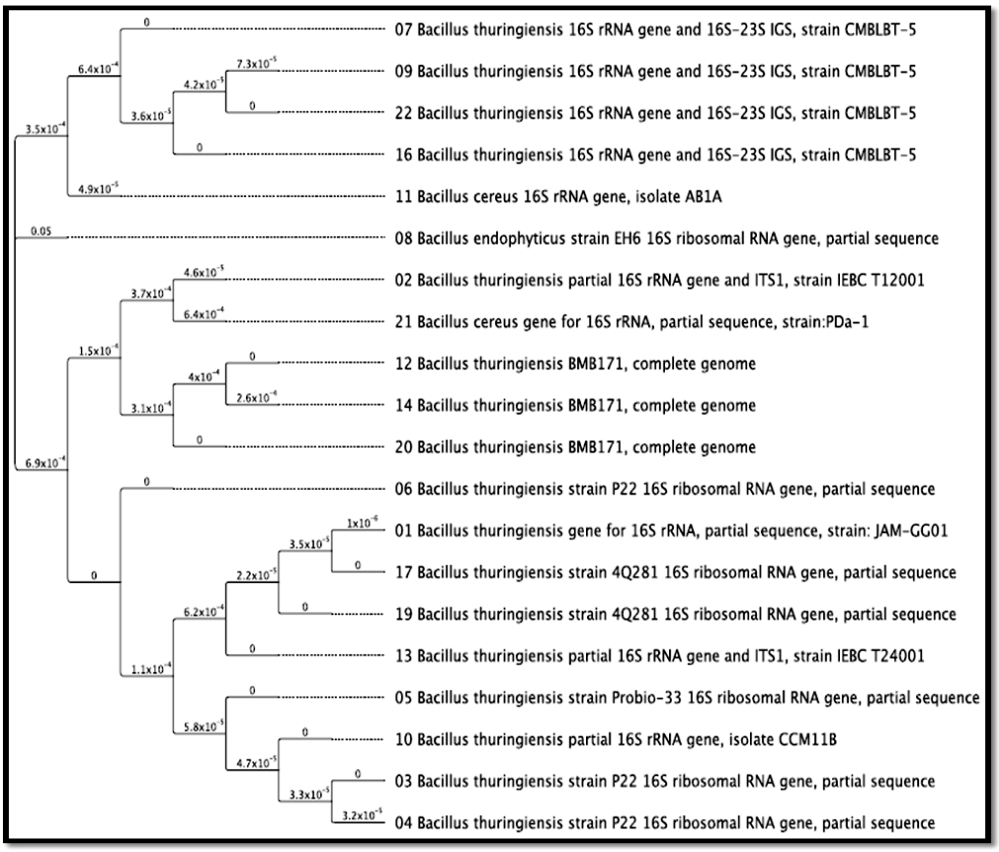


Figure 3. A dendrogram showing the relatedness of 16SrDNA of 15 native *Bt* isolates, two positive *BT* strains, two local *B. cereus* strains, and one local *B. endophyticus*.(N: 01: is the standard reference Bt H14 &02: is the standard reference BtKD1)



As an extension to our *Bt* research interests, part of the objectives of the research proposal here is to isolate additional *Bt* strains from different localities across the country, thereby creating a nucleus for the development of a large and diverse native *Bt* strain collection with a huge potential for control of a number of insects including mosquitoes. The *Bt* isolates collected will be identified and characterized biochemically in detail. PCR will be used to identify the type of the cry gene in local active isolates, as well as the cry protein before and after proteases activation, using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) analysis alongside the two available *B. thurengiensis* subsp. *kurstaki (BtkD1)* and *Bt israelensis* H-14 reference strains (Bukhari and Shakoori, 2010). The investigation and characterization of 130 kDa (Cry4A, B), 70 kDa (Cry4C, D) and 20 kDa (Cyt enhancer protein of Cry4) proteins, in mosquitocidal *Bt* isolates would not only provide baseline data for further studies on cry genes and their expression, but would also be helpful in isolating and screening for mosquitocidal *Bt*s worldwide. Lakxmy *et al.* (2011,) stated that periodical introduction of native strains of *B. thurengiensis* will add a new weapon in the armory for managing vector-borne disease and the management of insecticide resistance.

Another area of very promising research into biocontrol involves the Gram-negative bacteria *X. nematophilus* (*Xn*) and *P. luminescens* (*Pl*) that live in a mutualistic symbiosis with the entomopathogenic *Steinernematid* or *Heterorhabditid* nematodes, respectively (Forst, *et al*. 1997). Both *Xn and Pl* bacteria live symbiotically with these nematodes whose juvenile forms penetrate the cuticle of its insect host to inhabit the insect hemocoel (Forst, and Clarke, 2002). In the hemocoel, symbiotic bacteria are released from the nematodal gut into the haemolymph, where they reproduce and convert the body contents of the insect host into a nutrient soup that is ideal for nematode nutrition,

growth and reproduction. Finally, the nematodes reproduce and develop into new infective juveniles that are in turn infected by the symbiotic bacteria to form the new infective juvenile-bacteria symbiotic combination (Forst, and Clarke, 2002).

The exact biological role of *Pl* toxins in the infection process is still not clear. However, *Pl* toxins show toxicity against the insect host when administered either orally or by injection (Ffrench-Constant, *et al.* 2003). In contrast, however, Ffrench-Constant, *et al.* (2003) investigated the toxins that "make caterpillars floppy" (Mcf 1 and Mcf2) and the "*Pl* virulence cassettes", which are both only active *via* injection. Toxin Complexes (TCs) were identified as high molecular weight insecticidal complexes present in *Pl* strain W14 (Bowen, *et al.* 1998; Bowen, and Ensign, 1998). Four different TCs were isolated from nematodes and termed TCa, TCb, TCc, and TCd (Bowen, and Ensign 1998). These toxins disrupte the host insect midgut epithelium in a manner similar to that of the δ-endotoxins of *B. thurengiensis*. The corresponding gene loci (TCa, TCb, TCc and TCd) of these toxins were then cloned. Bowen, and Ensign (1998) have shown that each of these TCs can migrate as a single band on a non-denaturing gel; however, each complex is cleaved into numerous different polypeptides when run on a denaturing SDS-PAGE gel because each individual TC is encoded by a separate reading frame.

Like *Pl*, some *Xn* strains also showed toxicity when taken orally by their target host insect (Morgan, *et al.* 2001). The outer membrane and associated protein exposed on the surface of pathogenic bacteria are essential for recognition and interaction with the target host cells (Beveridge, 1999). In *Xn*, for instance, the outer membrane associated proteins are important for recognition by the bacterium to overcome the insect host immune responses and promote symbiotic association with the nematode host (Forst and Nealson, 1996; Leisman, *et al*., 1995). The larvicidal protein complex contained several major polypeptides ranging from 15 to 300 KDa (Khandelwal and Bhatnagar, 2003; Khandelwal, 2004). Oral infection with *Xenorhabdus* toxins showed toxic activity to the insect host (Morgan, *et al.*, 2001). Recently, Sheets, *et al*. (2011) analyzed the structure and stoichiometric composition of a TC from *Pl*, which is largely related to the TC from *Pl.* They found that native *Xenorhabdus* TC 1 is composed of three proteins (XptA2, XptB1 and XptC1), representing class A, B, and C proteins combined in a 4:1:1 stoichiometry. Using individual purified recombinant protein components of the *Xenorhabdus* and *Photorhabdus* TCs, they demonstrate that a fully active TC requires the presence of all three classes of A, B and C proteins, and that class B and C proteins from *Photorhabdus* (TCdB2 and TCcC3) can substitute for the B and C proteins from *Xenorhabdus* to form an active hybrid TC that has greater insecticidal activity than the native TC.

Two isolates from entomopathogenic nematodes belonging to *Steinernema* obtained from different regions in Egypt and Oman will be used in this study. The isolate from Oman has been classified as *Steinernema abbasi* (Elawad, *et al.*, 1997), which is symbiotic with *Xenorhabdus indica* (Tailliez, *et al.*, 2006). The isolate from Egypt has still to be investigated. Moreover, three isolates of entomopathogenic nematodes belonging to *Heterorhabditis* has been isolated from a different region in Egypt. *Heterorhabditis indicus* RM1 (El-Assal, *et al.*, 2002) and *Heterorhabditis sp* S1, which are symbiotic with *Photorhabdus akhurstii*.

Successful examples of field biocontrol trials

There have been successful applications of *B. thurengiensis*-based insecticides for over forty pest species in North America, as well as elsewhere in the world (Murty & Jamil, 1996). Formulations based on *B. thurengiensis* were registered with the EPA in USA as early as 1980. Commercial production commenced in USA and Europe in 1984 and

B. thurengiensis products were introduced to Australia in the same year (Clarke 1994; Murty and Jamil, 1996). *B. thurengiensis* has also been used in areas considered environmentally sensitive (Federici 1995; Federici *et al.*, 2003). In Africa, USA and Germany, these bacterial products have been used extensively in mosquito and biting fly control programmes. In Peru and Ecuador, Kroeger *et al*, (1995) reported the potential for malaria control with the biological larvicide *Bacillus thurengiensis israelensis* (*Bti*). In Western Kenya, Fillinger *et al*, (2003) reported efficacy and efficiency of *Bti* and *Bacillus sphaericus* formulations against *Anopheles* mosquitoes. In Malysia, Lee & Zairi (2006) have carried out a successful field evaluation of *Bacillus thurengiensis* H-14 against *Aedes* mosquitoes. More recently, in Italy, toxicity persistence of *Bti* was evaluated in laboratory and field trials to develop a new control measure for *Aedes albopictus* (Carrieri *et al*, 2009). In Swiss, Guidi *et al*, (2011) reported the distribution of *Bti* in soil of a wetland reserve after twenty-two years of mosquito control. We therefore, look forward to implementing a long-term strategy to apply the biological control strategy against mosquito vector in KSA using different biocontrol agents. Thus the current study constitutes the first step towards establishing this strategy using native *Bt* (isolated from the Saudi environment) to control mosquito vector.

In this project, the investigators aim to use the native isolates of symbiotic bacteria (*Xenorhabdus* and/or *Photorhabdus*) for characterization and purification of the TCs. Pathogenesis of the oral activity of TCs will be tested in rabbits (to assess its effect in mammals) as well as the target species of mosquitoes. Encouragingly, we currently have in hand one native isolates of *Bt* from the Saudi environment that showed a preliminary high toxicity against larval stages of both *Aedes* and *Culex*, the two mosquito species targeted by this study. The toxicity of different synergistic combinations of the target bacteria and the histopathological effects will also be investigated. This project will emphasize the importance of both investing efforts of isolating native Bt from almost every part of the world as documented in the literature and the discovery of several new Cry genes which may lead to the change of classical Cry genes nomenclature to the new ones (Crickmore et al., 1998). Also, the advantage of isolating novel Bt with modified/novel Cry genes will indeed help controlling the emergence of insect resistance problem, which will lay the groundwork for this new field in Saudi Arabia.

4. DESCRIPTION OF THE PROPOSED WORK

As described above, an evolving problem in KSA is the widespread occurrence of different species of mosquito vectors transmitting different types of life-threatening diseases. The Saudi Government is currently supporting strenuous efforts to control such diseases *via* control of their mosquito vectors. Because the use of broad-spectrum chemical insecticides not only leads to mosquito resistance but also to an environment contaminated with hazardous chemicals, there is an urgent need for alternative biocontrol agents in the battle against mosquito vectors in KSA. Hence, periodical introduction of native mosquitocidal bacterial strains should ensure a continuous method of biological control. This, in fact, could open a new chapter in the battle against mosquito-borne diseases threatening the lives of humans in KSA. Therefore, the current study is conducted to establish native mosquito larvicidal agent(s) against two widely distributed mosquito vectors in KSA, the *Cx. pipiens* and *Ae. caspius*. Native bacterial isolates of *B. thurengiensis* (*Bt*) and the nematodal symbiotic bacteria, *P. luminescens* (*Pl*) and/or *X. nematophilus* (*Xn*), will be considered in this study as they showed preliminary larvicidal effects in our labs. Therefore, the aim of this research project is to effectively participate in solving the serious public health problem of mosquito-borne diseases in KSA by using safe and effective larvicidal native bacteria. Thus, as an extension to our present mosquitocidal research interests, this study will focus on four steps to be investigated in the mosquitocidal native bacterial isolates.

Step I: Native *Bt* bacteria and their larvicidal effects.

Isolation of *Bt* strains from different localities across KSA will be carried out to produce a large and diverse native *Bt* strain collection with significant potential for discovering agents that are toxic to mosquito larvae. The biochemical characteristics and identity of these *Bt* isolates will be studied in detail. The potential mosquitocidal active *Bt* will be tested by PCR to investigate the type of the cry gene, as well as the cry protein before and after proteases activation, using SDS-PAGE analysis according to Bukhari and Shakoori, (2010). The 130 kDa (Cry4A, B), 70 kDa (Cry4C, D) and 20 kDa (Cyt enhancer protein of Cry4) proteins will be investigated as mosquitocidal toxins from native *Bt* isolates. This will not only provide baselines data for further studies on cry genes and their expression, but will also be helpful in isolating and screening of mosquitocidal *Bt*s worldwide.

Step II: Identifying Toxins of *Pl* and/or *Xn* bacterial isolates and their larvicidal effects

This part of the project will not only widen our screening to include bacterial symbionts isolated from mosquitocidal nematodes but also build a strong research collaboration with Egyptian institutions such as the National Research Center who are experts in this field. We will focus initially on native isolates of symbiotic bacteria *Xenorhabdus* and/or *Photorhabdus* from Egypt, for characterization and purification of their TCs. Pathogenesis of the oral activity of TCs will be investigated against the target mosquito larvae.

Step III: Effects of synergistic combinations of the larvicidal bacteria

Different synergistic combinations of potentially active bacteria/toxins will also be investigated to maximize the mosquitocidal effect (Promdonkoy, *et al.,* 2005 and Park, *et al.,* 2010). Synergistic effects of the mosquitocidal *Bt*, *Pl* and *Xn* isolates in different combinations on the *Aedes* and *Culex* mosquito larvae will be investigated. Isobolographic analysis will be carried out to dissect the synergistic combinations, and the determination of the degree of synergism will also be investigated as detailed in Sreshty *et al.,* (2011). The synergistic combinations that show higher toxicity will be considered and put forward as promising candidates to be used in the battle against mosquitoes in KSA.

Step IV: Histopathological effects of the larvicidal native bacterial isolates on larval midgut

The histological effects of bacterial isolates/toxins will be studied by histopathological investigation of the larvicidal effect on midgut epithelium of mosquito larvae, respectively. The effects of exposure to individual native *Bt*, *Pl* or *Xn* agents and combined mixtures will be investigated as detailed in Sreshty *et al.,* (2011). Mosquito larvae will be used for microtomy and permeabilization investigations after being exposed to LC_{50} concentrations of the larvicidal bacteria or their synergistic combinations specific to the mosquito species. Midgut sections will be prepared for examination under light, electron and/or confocal microscopy for determining the histological effect on midgut epithelium.

Principal Objectives:

We at King Saud University and our collaborators at the National Research Center in Egypt will focus on: **a**) Establishing the targeted mosquito colonies in the lab; **b**) isolating, identifying and characterizing native *Bt* isolates from the Saudi environment; **c**) isolating, identifying and characterizing the nematodal symbiotic *Pl* and/or *Xn* bacteria from the Egyptian environment, extracting, and purifying their larvicidal toxins; **d**) conducting mosquito larvicidal bioassays of individual bacteria/toxins as well as their synergistic combinations; and **e**) investigating the histopathological effect of the larvicidal bacteria/toxins and their synergistic combinations on larval midgut. Furthermore, this study constitutes a step towards: 1) overcoming mosquito resistance to insecticides, 2) participating in solving the serious public health problem of mosquito-borne diseases in KSA, 3) helping to keep the environment clean and safe and 4) ensuring periodical introduction of native mosquitocidal bacterial strains in the battle against mosquito-borne diseases in KSA. Finally, during this 2-years research project, we will work for establishing a laboratory dedicated to cutting-edge technology in the field of biological control as well as training young scientists in the skills required to ensure the continuality of this approach in order to develop and establish the field of biological control in KSA.

4.1 Approaches, tasks and phases:

Objective	Approach of achieving the objective
Establishment of mosquito colony in the lab	a) Collecting *Ae. caspius* larvae from Ehsaa' (eastern region of Saudi Arabia) b) Collecting *Cx. Pipiens* mosquitoes from Riyadh region (capital of Saudi Arabia). c) Identifying collected mosquito larvae using standard taxonomic keys (Wood *et al.*, 1979; Darsie & Ward, 2005). d) Rearing target mosquito species in the insectary of the Zoology Department, Faculty of Sciences, University of King Saud, under standard conditions as outlined in Ahmed *et al.*, (1999) to be ready for experimental purposes as needed.
Raising native larvicidal *B. thurengiensis* bacterial isolate(s).	***Bacillus thurengiensis* (*Bt*) isolates:** a) Isolation of native larvicidal strains of *Bt* isolates from the Saudi environment. b) Morphologically identifying the collected native *Bt* colonies according to Maheswaran *et al*., (2010). c) Morphological investigation of crystals and spores for potential native *Bt* isolate colonies as detailed in Tokcaer (2003). d) Confirmation of biochemical identity of native *Bt* isolates as detailed in Ichikawa *et al.*, (2008). e) Identifying the mosquitocidal–cry protein-genes of the native isolates by PCR and SDS-PAGE as detailed in (Ibarra *et al*, 2003). f) Screening of *Bt* isolates for larvicidal activity against *Ae. caspius* and *Cx. pipiens*. Native *Bt* isolates will be considered larvicidal if they cause > 50% mortality. The % corrected mortality will be calculated by the formula (X-Y/X × 100), where X = % of alive control larvae and Y = % of alive treated larvae. g) Preparation of the pure larvicidal *Bt* for bioassay as previously described (Dulmage *et. al*., 1970).
Raising entomopathogenic nematodes and their bacterial symbiont(s).	***Photorhabdus* and *Xenorhabdus* isolates:** a) Mass propagation of the entomopathogenic nematodes *Heterorhabditis sp*.S1 and *Heterorhabditis indicus* RM1 in the wax moth, *Galleria mellonella*, according to Dutcky, *et al.*, (1964). b) Isolating *Pl* and/or *Xn* symbiotic bacteria from their nematodes. c) Lab culturing of *Pl* and/or *Xn* under suitable growth conditions. d) Purifying and characterizing native toxin complexes of *Pl* and/or *Xn* according to Sheets, *et al.* (2011). e) Relative mass comparison of bacterial toxins by size exclusion chromatography. f) Electrophoresis analysis of toxins by SDS-PAGE. g) Measuring toxin binding by surface plasmon resonance using a BiaCore 3000 instrument. h) Measuring toxin binding to insect gut proteins by the method of Wolfersberger, 1993. i) Testing pathogenicity of toxin complexes to white New Zealand rabbits by oral administration.

Larvae toxicity bioassay (LC_{50} and LC_{90})	a) Performing larvicidal bioassays of each potential larvicidal bacterial agent against larvae of *Ae. caspius* and *Cx. pipiens* mosquito (Bukhari & Shakoori, 2010; Al-Roba *et al*, 2011 and Sreshty *et al*., 2011). b) Performing synergistic bioassays of bacterial combinations against larvae of *Ae. caspius* and *Cx. pipiens* mosquito according to the protocol of Sreshty *et al.,* (2011).
Histopathological effects of the larvicidal native bacterial isolates on larval midgut	Toxic histopathological effect of bacterial isolates on the larval midgut epithelium will be investigated as detailed in Sreshty *et al.,* (2011) and Abdelkefi-Mesrati *et al*,(,2011): a) Preparing treated larvae of *Ae. caspius* or *Cx. pipiens* 2h post-treatment with LC_{50} of potential larvicidal bacteria in 70% ethanol for preservation until used. b) Preparing treated larvae for microtomy and/or permeabilization. The resulting sections will be visualized and investigated under the microscope.

Form RE -D1-2

MAPPING OF PHASES AND TASKS TO ACHIEVE OBJECTIVES

Objectives	Phases	Tasks
Experimental elements	**Phase 1:** Task 1.1. (1st - 2nd month)	- Up-date- Literature review, data collection, setting up the lab and preparing chemicals, etc.
	Task 1.2. (3rd - 9th month)	- Field collection of bacterial isolates from various regions in Saudi Arabia and identifying *Bt* isolates.
	Task 1.3. (3rd – 10th month)	- Mass lab propagation of nematodes and isolation of *Pl* and *Xn* symbiotic bacteria.
	Task 1.4. (3rd -6th month)	- Field collection of mosquitoes and systematic identification of the target mosquito species.
	Task 1.5. (3rd -20th month)	- Lab rearing and maintaining of experimental mosquitoes. The 10th generations onward will be used for experiments in the next phases.
Raising native bacterial isolate(s)	**Phase 2:** Task 2.1. (4th – 12th month)	- Isolation of different native larvicidal strains of *Bt* from field collected samples.
	Task 2.2. (5th – 12th month)	- Extracting, identifying and characterizing endotoxin(s) from larvicidal *Bt* isolates.
	Task 2.3. (4th – 12th month)	- Isolation of *Pl* and *Xn* symbiont bacteria from their entomopathogenic nematodes.
	Task 2.4. (5th – 12th month)	- Extracting and identifying the toxins of *Pl* and *Xn*.
Larvae toxicity bioassay (LC_{50} and LC_{90})	**Phase 3:** Task 3.1. (10th – 12th month)	- Larvicidal bioassays of native *Bt* isolates
	Task 3.2. (12th – 14th month)	- Larvicidal bioassays of *Pl* and/or *Xn* symbionts
	Task 3.3. (14th – 16th month)	- Larvicidal bioassays of synergistic combination(s) of different forms of target bacteria.
Histopathological effects of the larvicidal native bacterial isolates on larval midgut	**Phase 4:** Task 4.1. (16th –18th month)	- Investigating histopathological effects of individual *Bt*, *Xn* or *Pl* on the larval midgut.
	Task 4.2. (18th –20th month)	- Investigating histopathological effects of synergistic combinations of target larvicidal bacteria/products on the larval midgut.
Reports and publications	**Phase 5:** Task 5.1. (21st –24th month)	- Writing reports, and preparing data/patent(s) for publication/registration.

Form RE -D1-3

4.2. Research Methodology:

4.2.1. Establishment of mosquito colony

The mosquito vectors, *Ae. caspius* and *Cx.* pipiens, have been selected for this study as they are widely distributed in most regions of the KSA. Mosquito larvae have been collected from the field and identified using standard taxonomic keys (Wood *et al.*, 1979; Darsie & Ward, 2005). Mosquitoes are now being reared in the insectary of the Zoology Department, Faculty of Sciences, King Saud University, under standard conditions as outlined in Ahmed *et al.,* (1999), to be ready for future experiments. Emerging adults are being maintained in rearing cages with permanent access to a 10% glucose solution (W/V). To maintain a colony stock of mosquitoes, the females are routinely fed upon the blood of an anesthetized mouse in order to lay eggs for new generations. The life cycle will be continued for at least 10 generations to ensure that the colony is free of any residual insecticidal or environmental pollutants. Larvae of this colony will be used for the experimental purposes of the current project. It is important to clarify that dealing with experimental mice is allowed by the Saudi low and does not need license.

4.2.2. Raising native bacterial isolate(s):

I) Identification of native *Bacillus thurengiensis* (*Bt*) isolates:

At least 200 to 300 isolates have been identified, using the *Bt* index, from 1000 samples from different localities in KSA. This forms an excellent nucleus for the development of a large and diverse native *Bt* strain collection with huge potential for the discovery of agents that will control local insects and/or provide possible novel biological anti-cancer cytocidal treatments. The following map of KSA shows the number of native *Bt* isolates from various regions collected by our Research Center in the Clinical Laboratory of Science, King Saud University (unpublished data). (Note: + means number of *Bt* isolates recovered from total number soil sample from the region). In the current project, sampling will include the same regions as well as the western, northern and eastern regions of mosquito distribution throughout the country.

These native *Bt* isolates have been collected from soil, lake water, the seacoast, debris and dead insects from different localities throughout Saudi Arabia. For isolation of *Bt*, the selective acetate enrichment process described by Travers *et al.,* (1987) or the modified Ethanol method of Koransky *et al*., (1978) and the recently developed Bukhari and Shakoori (2010) methods are used based on the type of sample collected.

Presumptive *Bt* colony morphology:

Plates will be checked extensively for any colonies that look to be identical to or close to the ideal form of *Bt.* The typical *Bt* colony morphology (Fig 4) is round, flat and white with regular or irregular margins, as described in Maheswaran *et al*., (2010). After each colony is purified on nutrient agar plates, it will be cultured and incubated for 48 h at 30°C before being processed.

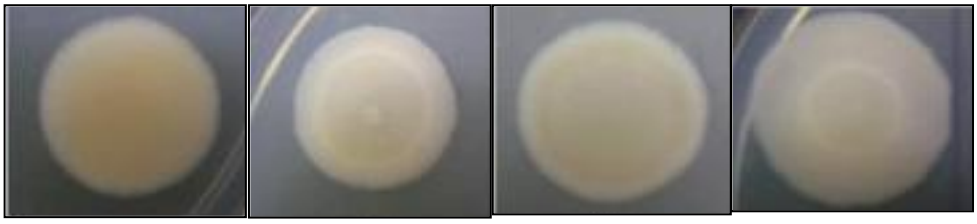


Figure (4) Different shapes of *Bt* colony morphology (Ashwini, 2006)

1. **Morphological investigation of crystals and spores:**

Potential *Bt* isolate colonies will be subject to examination by phase contrast microscopy for visible parasporal crystals next to the spores in the sporangium cells, as detailed in Tokcaer (2003).

2. **Biochemical reactions and identification of *Bt***

All *Bt* native isolates recovered from the examined samples will be confirmed for their biochemical identity as detailed in Ichikawa *et al.,* (2008) using API 50 CH and API 20 kits (BioMérieux, Marcyl Etoile, France), according to the manufacturer's instructions.

3. ***Bt* bacterial samples preparation for PCR:**

Bt isolates (with approved potential larvicidal activity against mosquito larvae) will be tested to identify the mosquitocidal–cry protein-gene content of the native isolates by PCR as detailed in Ibarra *et al*, (2003). The primers from conserved regions of the cry genes that are specific for larvicidal activity are illustrated in the following table showing characteristics of the universal primers that will be used.

Primer pair	Sequence	Positions	Gene(s) recognized	Product size	GenBank accession no.	References
Cry2	(F) 5-GAGTTTAATCGACAAGTAGATAATTT-3 (R)5-GGAAAAGAGAATATAAAAATGGCCAG-3	531–1057	*cry2Aa*	526	M31738	Ibarra et al, 2003
		376–902	*cry2Ab*	526	M23724	
		2500–3020	*cry2Ac*	520	X57252	
		1041–1541	*cry2Ad*	500	AF200816	
Cry4	(F) 5-CGTTTTCAAGACCTAATAATATAATACC-3 (R) 5-CGGCTTGATCTATGTCATAATCTGT-3	1868–2189	*cry4Ba*	321	X07423	Ibarra et al, 2003
Cry11	(F) 5-CGCTTACAGGATGGATAGG-3 (R) 5- GCTGAAACGGCACGAATATAATA-3	990–1332	*cry11Aa*	342	M31737	Ibarra et al, 2003
		1025–1368	*cry11Ba*	342	X86902	
		1048–1400	*cry11Bb*	452	AF017416	
Cry32	(F) 5-TGGTCGGGAGAGAATGGATGGA-3 (R) 5-ATGTTTGCGACACCATTTTC-3	2236–2913	*cry32Aa*	677	AY008143	Ibarra et al, 2003
		2338–3014	*cry32Ba*	676	BAB78601	
		2254–2930	*cry32Ca*	676	BAB78602	
		2218–2894	*cry32D*	676	BAB78603	
Cyt1	(F) 5-CCTCAATCAACAGCAAGGGTTATT-3 (R) 5-TGCAAACAGGACATTGTATGTGTAATT-3	197–674	*cyt1Aa*	477	X03182	Ibarra et al, 2003
		85–565	*cyt1Ab*	480	X98793	
		97–574	*cyt1Ba*	477	U37196	
Cyt2	(F) 5-ATTACAAATTGCAAATGGTATTCC-3 (R) 5-TTTCAACATCCACAGTAATTTCAAATGC-3	509–865	*cyt2Aa*	356	Z14147	Ibarra et al, 2003
		529–884	*cyt2Ba*	355	U52043	
		649–1004	*cyt2Bb*	355	U82519	
		196–551	*cyt2Ca*	355	AAK50455	

Bt strains will be cultured for 12 hours on a nutrient agar plate. A loopful of cells will be transferred to 0.1 ml of water, frozen for 20 min at -70°C, and thereafter boiled for 10 min in water to lyse the cells. Cells will be briefly spun (10 s at 10,000 rpm), and 15 µl of supernatant used as DNA template in the PCR.

PCR primer sequences used for this experiment are shown in the Table presented above. PCR amplification will be performed as detailed in Guo *et al*, 2008 with initial denaturation at 94C° for 5 min, followed by 30 cycles of denaturation at 94°C for 1 min, annealing for 1 min and extension at 72°C for 1 min, with a final extension at 72°C for 5 min. Then 15 µl of the sample will be run on a 2% agarose gel for DNA band separation (Cero´n, *et al*,1994; Ibarra *et al*, 2003 and Ashwini, 2006). A positive control(s) will be run in parallel using the available standard strains of *B. thurengiensis* subsp. *kurstaki* (cry 2) and the *Bti* H-14 (cry 4, cry 11, cyt1 and cyt 2) as well as tracking Kits DNA ladder for band size determination (Ben-Dov *et al*,1997).

4. Screening of *Bt* isolates for larvicidal activity

The colonies that resemble *Bt* and are positive for parasporal crystal inclusions (Jouzani *et al*, 2008) by staining and phase-contrast microscopic examination will be subjected to tests for larvicidal activity. For initial screening, 20 4th instar larvae of *Ae. caspius* or *Cx. pipiens* will be placed in a waxed paper cup containing concentrated spore-crystal suspension (1×10^5 to 1×10^7) of test organism suspended in 100 ml chlorine-free tap water. The mortality of these test larvae will be recorded after 24 and up to 48 hours of continuous exposure. A native *Bt* isolate will be considered larvicidal if it causes >50% mortality. Cups of control larvae will receive no bacteria. The % corrected mortality will be calculated by the formula X-Y/X × 100, where X = % of alive control larvae and Y = % of alive treated larvae.

5. Preparation of larvicidal *Bt* for bioassay

For laboratory production of larvicidal *Bt*, the pure active isolate will be inoculated into 250-ml Erlenmeyer flasks containing 50 ml sterile TYG broth. The flasks will then be incubated on a gyratory shaker (G-52 New Brunswick Science Co.) at 180 rev/min at 28-30°C. Six-hour-old cells (3% inoculation) will be transferred three successive times in TYG broth to obtain synchronously dividing cells. This will then be used to seed a solid fermentation medium in Petri dishes consisting of 15 g wheat flour, 10 g glucose, 5 g yeast extract, 0.1 g KH_2 PO_4-$7H_2O$, 0.5 g $MgSO_4$ H_2O, 3 g NaCl, 0.1g $FeSO_4$, 5 g Protease Peptone, and 25 g Agar, made up to 1 liter in distilled water. The pH is adjusted to 7.2 before autoclaving at 121°C for 30 min. After incubation for 72h at 30°C, the cells are then harvested by scraping with sterile scalpel and suspended in 5% lactose solution. The spore-parasporal inclusion complex will be precipitated as previously described (Dulmage *et. al*., 1970). After sterile-water washing of the putative purified parasporal inclusions by centrifugation, they are then lyophilized and kept until needed for larvicidal bioassays.

II) *Photorhabdus* (*Pl*) and *Xenorhabdus* (*Xn*) isolates:

1. Nematodal propagation and isolating and culturing symbiotic bacteria:

Two native isolates of *Photorhabdus* spp. will be used in this study. They will be isolated from two species/strains of entomopathogenic nematode, *Heterorhabditis sp*. S1 and *Heterorhabditis indicus* RM1. These nematodes originated from Egyptian soil, and will be mass-propagated in the wax moth, *Galleria mellonella*, according to Dutcky, *et al.,* (1964). Two X*enorhabdus spp*. will be isolated from two nematode species, one of which is *S. abbasi*, originally from Oman, and the other *Steinernema sp*. SII, originally from Egypt. Stocks will be maintained on petri plates containing 2% proteose-peptone no. 3 (PP3) and 1.5% agar (Difco Laboratories, Detroit, MI). The cultures will be incubated at 30°C for 3 days and will then stored at room temperature, and transferred at monthly intervals. Primary-formed colonies will be selected on the basis of colony morphology, bioluminescence, pigmentation and identity of their 16srDNA. Inclusion protein production will be inoculated into 1-liter flasks containing 200 ml of 2% PP3 broth supplemented with 0.5% polyoxyethylene sorbitan monostearate (Tween 60; Sigma Chemical Co., St. Louis, MO). Cultures will be incubated for 48 h at 30°C on a rotary shaker at 250 rpm.

2. Purification and characterization of native toxin complexes:

Toxin purification and characterization from *Pl* or *Xn* will be carried out according to Sheets, *et al.* (2011) with some modifications; pellets obtained from 2 liters of culture after overnight incubation of the *Xn* or *Pl* bacteria will be suspended in 50 mM Tris-HCl pH 8.0, 100 mM NaCl, 1 mM DTT, 10% glycerol and lysozyme (0.6 mg/ml). A small amount of glass beads (0.5 mm diameter) will be added and cells will be disrupted by sonication. Broken cells will then be centrifuged at 48,000 × g for 60 min. At 4°C, supernatant will be collected, a bacterial protease inhibitor cocktail will be added (Sigma, St. Louis), and the solution will be dialyzed against 25 mM Tris-HCl, pH 8.0 overnight. The protein will then be loaded onto a Q Sepharose XL (1.6 × 10 cm) anion exchange column. Bound proteins will be eluted using a linear 0 to 1 M NaCl gradient in 10 column volumes. The high molecular weight TCs will be eluted in the early fractions and will be concentrated and loaded onto a Superose 200-size exclusion column (1.6 × 60 cm) (Pharmacia) using 50 mM Tris-HCl, 100 mM NaCl, 5% glycerol, 0.05% Tween-20, pH 8.0. The large molecular weight proteins eluting from the column will be brought to 1.5 M ammonium sulfate concentration and will be loaded onto a phenyl Superose (0.5 × 5 cm) hydrophobic-interaction column. Proteins will be eluted using a decreasing linear gradient of 1.5 to 0 M ammonium sulfate in

25 mM Tris-HCl, pH 8.0, over 20 column volumes. The TCs will be eluted together as a broad peak at low salt concentration. The proteins will be dialyzed overnight against 25 mM Tris-HCl, and will be loaded onto a high resolution MonoQ (0.5 × 5 cm) anion exchange column. Two separate TCs will be resolved with baseline resolution using a linear gradient of 0 to 1 M NaCl in 25 mM Tris-HCl obtained in 20 column volumes. The proteins will be identified by N-terminal amino acid sequencing and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry analysis. Purification of recombinant XptA2, co-expressed XptB1 + XptC1, and TcdB2 + TccC3 will be done using similar chromatographic procedures. Purified toxins will be used for the other relevant experimental use.

3. Relative mass comparison by size exclusion chromatography:

XptA2 (0.5 mg/ml) will be incubated overnight at 4°C with: 1) XptB1 + XptC1 (0.5 mg/ml each) in running buffer consisting of 25 mM Tris-HCl pH 8.0, 5% glycerol, 0.05% Tween-20; or 2) an equal volume of running buffer only (control). To subsequently separate XptA2 from the unbound B and C proteins, the mixtures will be applied to a Superdex 200 10/30 gel filtration column (AP Biotech, Piscataway, NJ).

4. Electrophoresis:

Analysis of proteins will be done by SDS-PAGE using 4-20% Tris-Glycine polyacrylamide gels (BioRad, Hercules, CA). Native-PAGE will be conducted for the electrophoretic mobility shift assays, using precast NuPAGE® Novex 3-8% Tris-Acetate gels (Invitrogen, Carlsbad, CA) at 150 volts for 3 h.

5. Surface plasmon resonance:

The binding of proteins will be measured by surface plasmon resonance using a BiaCore 3000 instrument (available in the Egyptian site). Briefly, the proteins will be immobilized onto the surface of a dextran/gold CM-5 or CM-4 Biacore chip following the manufacturer's recommended amine coupling procedure employing 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS). Remaining free reactive esters will be blocked with ethanolamine. For the analysis, the buffer flow rate will be adjusted to 30 µl/min, using HEPES Buffer: (NaCl, EDTA, Surfactant P20, (HBS-EP) (BiaCore). Association of XptB1-XptC1 with the immobilized XptA2 will be measured for 200 seconds, and dissociation also will be measured for 200 seconds by flowing buffer in the absence of XptB1-XptC1 protein over the immobilized XptA2. A "blank" surface will be prepared using EDC and NHS and blocking with ethanolamine using the same procedure as describe above, but without any protein. Signals from the "blank" surface will be subtracted from the signal from the surface containing the immobilized proteins.

For measurement of binding to insect gut binding proteins, brush border membrane vesicles will be prepared by the method of Wolfersberger, 1993, with some modification from last instar *Galleria mellonella* insect larvae. The vesicles will be solubilized with CHAPS detergent (2% final) in 20 mM Tris, pH 7.5, 1 mM EDTA, 1 mM MgSO4, 0.01% NaN3, and 10% glycerol with protease inhibitors. Following 1-h gentle mixing at 4°C, the mixture will be centrifuged for 1 h at 100,000 × *g* at 4C, the supernatant collected, filtered through a 0.2-m membrane and loaded onto a MonoQ (0.5 cm diameter, 5 cm length) anion exchange column (Pharmacia) equilibrated in solubilization buffer containing 1% CHAPS. The proteins will be eluted with a linear gradient from 0 to 500 mM KCl in solubilization buffer containing 1% CHAPS. Samples will be kept at 4°C and used on the day of preparation. XptA2 will be immobilized on a CM-5 chip and each fraction tested for binding. The fraction

showing the strongest binding will then be immobilized onto a CM-4 chip and various concentrations of XptA2 passed over to measure binding.

6. **Toxin complexes pathogenicity to rabbits:**

White New Zealand rabbits will be used for oral application of TCs derived from *Xn* or *Pl* bacteria. This experiment will be carried out in four groups, three groups for oral applications and one group for control. Every group will contain three rabbits. Blood samples will be obtained after time intervals (2, 4 and 8 days post-treatment) for blood haemocyte count and cell differentiation. The treated groups of rabbits will be slaughtered and dissected for liver, kidney and lung histopathological studies and will be compared with control. This part will be carried out in Egypt in where dealing with experimental animals is allowed by the Egyptian low and does not need license.

4.2.3. Testing mosquito toxicity bioassay (LC_{50} and LC_{90})

I) Individual larvicidal bioassay

The idea of using such new local bacterial isolates against mosquitoes is advantageous for two main reasons. First, cross-resistance conferred by insecticide resistance mechanisms seems unlikely to occur (Ffrench-Constant, *et al.*, 2004). Second, this bacterial product is a cheap, safe and effective alternative to conventional pesticides for mosquito vector control in KSA. Larvicidal activity of the potentially positive *Bt* and *Pl* candidates in the current study will be tested against the third larval instar of the target mosquito vectors, *Ae. caspius* and *Cx. Pipiens*, and calculating LC_{50} and LC_{90} will be carried out as described previously (Bukhari & Shakoori, 2010; Al-Roba *et al*, 2011 and Sreshty *et al.*, 2011). All bioassays are to be conducted at 28 ± 2°C and about 80% relative humidity. The mortality of the larvae will be determined after 24 to 48 hr of continuous exposure.

II) Synergistic bioassay

This part of the study aims to exploit the benefits of synergism between the larvicidal *Bt*, *Pl* and *Xn* isolates by evaluating the toxicity of different combinations of mixtures of toxins to the targeted *Aedes* and *Culex* mosquito larvae. Isobolographic analysis will be performed to distinguish the synergistic combinations of *Bt*, *Pl* or *Xn*, followed by determination of the degree of synergism through synergy and improvement factors, as detailed in Sreshty *et al.*, (2011). This is because synergistic combination has been shown to improve the toxicity of less-toxic biocontrol agents (Promdonkoy, *et al.*, 2003, Park, *et al.*, 2010 and reviewed by Gurr and Kvedaras, 2010). The synergistic combination(s) that show higher toxicity will be evaluated and suggested as the eventual candidate(s) to be used for controlling mosquitoes in KSA.

4.2.4. Histopathological effects of bacterial isolates/toxins on larval midgut

The histopathological effects of the native larvicidal bacteria or their synergistic combinations on the larval midgut epithelium and muscles will be investigated:

A) Investigating the effect on midgut epithelium

Cytological effects of larvicidal bacteria or their synergistic combinations on the larval midgut will be investigated. Mosquito larvae will be treated with the LC_{50} of the potentially effective larvicidal bacteria or their synergistic combinations. Larval midguts will be dissected prior to death (18-20 h post-treatment) and

will be routinely fixed. Thin or ultrathin sections will be stained for light or electron microscopy, as described by Villalon *et al*., (2003) and Ahmed *et al.,* (2011), or Bauer and Pankratz, (1992), respectively.

B) Investigating the speed of effect on midgut muscles

I) Preparation of the mosquito specimens

As detailed in Sreshty *et al.,* (2011), third instar larvae of *Ae. caspius* and *Cx. Pipiens* mosquitoes will be exposed to LC_{50} concentrations of *Bt*, *Pl* or *Xn* and their mixture of synergistic combination specific to the target mosquito species in this study. After 2 h, larvae will be collected from the treatment trays and washed in 1× phosphate buffered saline (PBS) to remove the debris attached to their bodies. The specimens will then be transferred into 70% ethanol for preservation until used.

II) Microtomy and permeabilization

As detailed in Sreshty *et al.,* (2011), treated mosquito specimens will be dissected under a light microscope for removing the midgut. Midguts will then be frozen in TBS tissue-freeze medium (Triangle Biomedical Sciences, Durham, NC) and sectioned into 10-µm sections. These sections will be mounted on clean glass slides and fixed with 4% paraformaldehyde solution for 10 min. Slides will then be incubated for 2–3 min in 0.1% Triton X-100 for permeabilization.

III) Light microscopy

Sections will be investigated under light and/or confocal microscopes. Images will be processed and analyzed using suitable image processing software according to Sreshty *et al.,* (2011). This part will be carried out at King Faysal Specialized Hospital in Riyadh.

4.2.5. Statistical analysis

All statistical analyses for this study will be undertaken using MINITAB software (MINITAB, Stat College, PA, v. 13.1, 2001) where appropriate. Data will first be tested for normality and variance homogeneity prior to any further analysis with the suitable test. In the case of non-parametric data, the Kruskal-Wallis test will be used to determine the overall effects of treatments prior to the individual comparisons using the Mann-Whitney *U* non-parametric test. In case of parametric data, the ANOVA or t-test will be used based on the nature of the target parametric data.

4.3. MANAGEMENT PLAN.

ROLE AND INVOLVEMENT DURATION OF RESEARCH TEAM

Team Members	**Role**	**Duration** (months)
Senior Personnel: **Dr Ashraf M. Ahmed**	**Directing and planning** Will be the principal investigator who will manage the project work plan and coordinate with all project participants. Also will be responsible for: • Field collection and identification of mosquitoes. • Helping in designing experiments and directing the research team members. • Rearing, identifying and maintaining the target mosquito species in the lab. • Supervising/carrying out the larvicidal bioassays and histological experiments in the lab. • Guiding the research assistants and supervising post-graduate students and technicians. • Solving any problems encountered. • Arranging for preparation of all reports, analyzing data and preparing manuscripts for publication.	**24**
COI-1: **Prof. Talat A. M. El-Kersh**	Will be responsible for *Bt* relevant work summarized as: • Field collection of various Saudi *Bt* local samples including those from soil, water, dead insects (adult and larvae) at rearing localities, phylloplanes, and on herbivores and dried and fresh feces. • Processing collected samples of native *Bt* strains including isolation, culture enrichment, purification, and identification. • Investigating phase contrast microscopy and staining for parasporal crystal formation of recovered *Bt* isolates. • Follow-up the maintenance of active *Bt* isolates in 15% glycerol at -80 deg. C as well as in soft TSA media. • Preparing *Bt* for the relevant bioassay experiments, • Characterization of potential *Bt* isolates. • Investigating crystal morphology by electron microscopy and other methods. • Performing PCR cry gene determination in relation to delta-endotoxin production and its biological spectrum. • Ensuring the continuous availability of *Bt* isolates in adequate amounts for larvicidal bioassays. • Helping in analyzing the data and writing reports and manuscripts for publication.	**20**
COI-2: **Dr Tahany H. Ayaad**	Will be responsible for conducting experiments related to nematodal symbiotic bacteria/toxins summarized as: • Supervising/performing nematodal cultures in the lab. • Supervising/performing isolation of nematode symbiotic bacteria. • Supervising/performing extraction and characterization of bacterial toxins • Ensuring the continuous availability of bacterial toxins in adequate amounts for larvicidal bioassay. • Helping in performing larvicidal bioassays and histological experiments. • Helping in analyzing the data and writing reports and manuscripts for publication.	**20**

Other Personnel: Research assistant	One research assistant who will be responsible for: • Preparation of reagents and solutions. • Help in collecting mosquitoes and bacteria from the field under the direction of the PI and COI-1. • Help in performing lab experiments • Help in supervising and guiding postgraduate students • Help in analyzing the data, writing reports and publications	**20**
Postgraduate students: **a) MSc:** Sultan A. Alharbi, number: 430101290 KSU. **b) PhD**: Hosain M. Al-Qahtany, number 429106238, KSU	Will perform the following duties under the supervision of the relevant researchers in their labs: • Help in collecting mosquitoes and bacteria from the field. • Help in rearing mosquitoes in the lab. • Help in preparing growing media and mass propagation of bacteria and nematodes. • Help in preparing buffers, other chemical solutions and keeping the lab(s) clean and well organized. • Performing some of the designed experiments.	**20**
Consultant: **a)** Prof. H. I. Hussein	The project will benefit from the participation of Professor Hamdy Ibrahim Hussein (Dept. of Plant Protection, College of Food Sciences and Agriculture, King Saud University); he will be consulted regarding toxicity tests, and adjusting the initial lethal and sub-lethal doses. He will also help in supervising postgraduate students, analyzing data and preparing data for publication.	**10**

Form RE -D1-4

PROJECT WORK PLAN

PHASES & TASKS	INVOLVEMENT DURATION	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
PHASE I																									
Task 1.1: Reading, data collection and lab management	A. Ahmed, T. Ayaad T. El-Kersh postgrad. student & Secretarial																								
Task 1.2: Field collection and identification of *Bt* isolates	T. El-Kersh, Research assistant & Worker																								
Task 1.3: Rearing nematodes and isolation of *Pl* and *Xn* bacteria.	T. Ayaad & Technician																								
Task 1.4: Field collecting and identifying of mosquitoes	A. Ahmed, postgrad student & Worker																								
Task 1.5: Lab rearing and maintaining of experimental mosquitoes.	A. Ahmed, postgrad student & Technician																								
PHASE 2																									
Task 2.1: Isolation of native larvicidal *Bt* from field collected samples.	T. El-Kersh & Research assistant																								
Task 2.2: Extracting, identifying and characterizing *Bt* endotoxin(s)	T. El-Kersh & Research assistant																								
Task 2.3: Isolation of *Pl* and *Xn* symbiont bacteria from their nematodes	T. Ayaad & Research assistant																								
Task 2.4: Extracting and identifying toxins from *Pl* and *Xn*.	T. Ayaad & Research assistant																								
PHASE 3																									
Task 3.1: Larvicidal bioassay of native *Bt*	A. Ahmed & postgrad student																								

Task 3.2: Larvicidal bioassay of *Pl* and *Xn* toxins	A. Ahmed & postgrad student																								
Task 3.3: Larvicidal bioassays of synergistic combinations	A. Ahmed & postgrad student																								
PHASE 4																									
Task 4.2: Histopatholo-gical effects of *Bt*, *Xn* or *Pl*. on larval midgut	A. Ahmed, T. Ayaad & postgrad student																								
Task 4.2: Histopatholo-gical effects of synergistic combinations on larval midgut	A. Ahmed, T. Ayaad & postgrad student																								
PHASE 5																									
Task 5.1: Preparation of data for publication and final report	A. Ahmed, T. Ayaad, T. El-Kersh, Research assistant & secretarial																								

Form RE- D1-5

4.4. PROJECT DELIVERABLES: Measures of successful outcomes; RELATIONSHIP TO STRATEGIC FRAMEWORK

PROJECT EXPECTED OUTCOMES	STRATEGIC TECHNOLOGY PROGRAM GOALS				PROJECT OBJECTIVE ACHIEVED
	Development and retention of national manpower and expertise in medical and health sciences research	**Development of infrastructure for sustainable, cutting-edge and competitive research in the medical and health sciences**	**Facilitate the performance of novel, competitively funded and high quality research in the medical and health sciences**	**Effective communication of research findings and significance of those findings to policy-makers and the public**	
Knowledge of the mechanisms of isolation, identification and characterization of native larvicidal bacteria.	Providing opportunity for training students, scientists and technicians on isolating, raising and characterizing native mosquitocidal bacteria.	This will establish a biological control laboratory, and research collaboration between medical entomology and mosquito-borne disease control in the field of health science.	This will be of high impact for the use of novel effective and safe biocontrol measures of mosquito-borne diseases in KSA.	Encourage of establishing an environmentally safe biocontrol measures against disease vectors is of interest to the KSA government.	**Raising native larvicidal *B. thurengiensis* bacterial isolate(s).**
Knowledge of raising nematodes, isolating their symbiotic bacteria and extracting their toxins.	Providing opportunity for training students, scientists and technicians on isolating, raising and characterizing native mosquitocidal nematodes, and transfer this technology from Egypt to KSA	This will further establish competitive research ideas about safe biocontrol agents against disease vectors, and hence, mosquito-borne diseases as well as relevant human nematodal diseases.	This will be of high impact on high quality research for utilizing safe nematodes and bacterial toxins in biocontrol measure for eradicating public health problems *via* high quality research.	This will be useful for the application of novel biocontrol measures for mosquito-borne diseases that establish communication between different relevant research areas for the benefit of public health	**Raising entomopatho-genic nematodes and their bacterial symbiont(s).**
Knowledge of producing bacterial products for controlling and mosquito-borne diseases.	Providing opportunity for training students, scientists and technicians on the use of biocontrol agents against mosquito vectors.	This will develop a novel biocontrol measure of mosquito vectors and hence mosquito-borne diseases. This could establish new area of medical researches	This could enhance research for the use of safe and effective larvicidal bacteria to control mosquito-borne diseases.	This is important for the limitation of mosquito-borne diseases as a real response to the call of the KSA government.	**Larvae toxicity bioassay (LC_{50} and LC_{90}).**
Knowledge of the histopathological effects of the native bacteria on mosquito larvae midgut.	Providing information about the mechanism of bacterial gut toxicity mechanism and strength.	Developing ideas about maximizing the effects of biocontrol agents against insect vectors and hence improving public health	This would enhance the applications of safe bio-mosquitocidal control to ensure public health stability.	This could direct the attention of policy-makers towards the biocontrol limitation of mosquito-borne diseases.	**Histopathological effects of the larvicidal native bacterial isolates on larval midgut.**

Form RE- D1-6

5. VALUE TO THE KINGDOM OF SAUDI ARABIA (KSA)

1. This study will potentially be of great value to all stakeholders in the field of biocontrol of insects in the KSA. Not only is biocontrol safer for the environment, livestock and humans but it will also be a vital component in overcoming insect resistance to commonly used chemical insecticides.

2. Once an effective novel larvicide is produced from *Bt*, *Pl* or *Xn* and/or any of their products, it will be of great help in initiating future effective biocontrol measures against mosquito vectors in the KSA. It will be of particular value in controlling dengue fever and similar mosquito-borne diseases, which threaten not only the millions of pilgrims who visit the western region of KSA every year but also the community throughout the country and of countries surrounding Saudi Arabia.

3. The outcome of this project could form the basis for future strategies aiming at establishing a Research Centre, Research Chair or laboratory dedicated to the development of future long-term work on biological control of insect vectors to reduce the chance of spreading deadly infectious diseases within the Saudi community.

4. This project will establish a fruitful long-term collaboration between researchers from KSA and Egypt, allowing exchanging and transfering knowledge and experiences between the two countries. To summarize, the value of the project is first in providing the resources necessary (mosquito colony, strain collections of the relevant bacteria and knowledgeable expertise of the applicants).

5. This work could lead to the patenting of commercial bacterial products for mosquito control.

6. Future approaches:

a) Although this project will initially produce results on a laboratory scale, its future application on a large scale could be possible for adaptation to industrial production with sponsorship by governmental agencies, such as the Ministries of Health, Finance and/or Agriculture. We believe in the encouraging outcomes of this study, if further funding is available, so as to enable us to plan for a future semi-field application, according to Chui *et al.,* (1995), Gunasekaran *et al.,* (2004) and Kim *et al.,* (2006), and based on the recommendations of WHO (2006) on this behalf . This may lead us to conduct a filed integrated mosquito bio-control measure in KSA.

b) This research provides an opportunity for graduate students to initiate their research proposals for obtaining masters and doctorate degrees in this area of advanced and applied research directions in microbiology, entomology, and medical biotechnology and their industrial applications.

c) Previous extensive screening of *Bt* Cry proteins showed novel biological activities other than entomopathogenicity (Ohba and Aizawa, 1986; Hastowo *et al*, 1992; Mizuki *et al*, 1999;Mizuki *et al*., 2000; Maeda *et al*, 2000; Kondo, et al ,2002;Lee *et al*, 2003; Xu *et al* ,2004; Ito *et al*, 2004; Okumura *et al*., 2005 & 2006; Yamashita, *et al*, 2005; Katayama, *et al*, 2005; Cappello, *et al*, 2006;Choi *et al*, 2007; El-Sadawy et al ,2008; Cahan, *et al*, 2008; Ohba *et al*, 2002 & 2009, Yan Hu, *et al*, 2011). Recently, unique parasporin proteins that preferentially target human cancer cells have been discovered (Kitada et al,2006; Nagamatsu, *et al*, 2010; Abou El-Hag and Safhi, 2011). These observations identified a new group of *Bt, B. thurengiensis* serovar dakota (H15), that may have anticancer activity and revealed the possibility of new applications in the medical field. Thus, future research projects are to be conducted in the near future for utilizing these kinds of bacteria in the battle against the rapidly evolving breast cancer problem in KSA (Saudi Ministry of Health, 2009

6. PROJECT EXECUTION.

6.1. CURRENT RESOURCES: Instruments

- Multiphor II. Horizontal gel electrophoresis unit (Isoelectric Focusing)
- Hoefer Gel Eluter GE 200 (for elution of protein from gel)
- UV/Visible spectrophotometer
- Perkin Elmer HPLC Series 200 (Fully automated)
- Hoefer SE 600 Ruby Vertical gel electrophoresis unit
- Freeze dryer, and Ultrafreezer (-80°C)
- Two (2) laminar flow cabinets
- Inverted and Microscopes, Olympus equipped with Phase contrast, dark-field, and camera
- Ultracentrifuge, with swinging rotors
- Laminar Flow – Vertical 700 ASALAIR and Biolgical Safety Cabinet – Class 2
- Shaking water bath - Memmert
- Transmission and scanning electron microscopes
- Three (3) refrigerators
- PCR setup and necessary accessories for RT-PCR and Gel Documentation System
- Mosquito rearing room (Insectary)

6.2. REQUESTED RESOURCES:

A) Materials and Consumables:

- DNA Extraction kits and Plasmid DNA Extraction Kits
- Universal and specific primers
- SDS/PAGE materials for proteins and DNA cry genesagarose gel electrophoresis
- Required accessories, track dyes, molecular wt standards, buffers
- Culture media, yeast extract, LB, BHI, TSA, with additives, Packed RBCs
- Antibiotics discs for susceptibility patterns of *B. thurengiensis*
- Glassware EM flasks, 125, 250, 500, and 1000 ml
- RPMI 1640 culture media
- Fetal calf serum
- API CH 50 kits, Gram(+) *Bacilli*-identification
- Supplementary API 20 kits
- CCl4, acetone, solvents, phenol, CHCl3
- Na2SO4, NaBr, Poly E G 6000, Sucrose
- Cesium chloride for Crystal/spore separations
- Osmium Oxide, Glutaraldehyde, Buffers for SEM of crystals
- Sera Kits for *B. thurengiensis* serotyping
- Gloves, disposable pages
- Glycerol, Lactose
- Sonicator for crystal/spore separation
- Protease Peptone no. 3 (PP3), Nutrient broth
- Nutrient Agar, Lb media and Muller Hinton Agar and Blood agar base, MHA media
- Polyoxyethylene sorbitan monostearate (Tween 80, 60, 40, and 20)
- srDNA kits and 18 srDNA kits
- Glycerol binuclear
- Nuclear free water
- Polyacrylamide gel electrophoresis and relevant chemicals
- Dialysis bags low molecular weight
- Protein markers kits

- mM Tris-HCl pH 8.0 and 1 mM DTT (dithiothritol)
- Glycerol
- Lysozyme and bacterial protease inhibitor cocktail
- Q Sepharose XL (1.6 × 10 cm) column, Superose 200 size (1.6 × 60 cm) column, MonoQ (0.5 × 5 cm) column, N-terminal amino acid, Superdex 200 10/30 gel filtration column, dextran/gold CM-5/CM-4 Biacore chip, 3-dimethylaminopropyl, 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC), Ethanolamine and N-hydroxysuccinimide (NHS)
- HPS-EP buffer (BiaCore), EDTA, MgSO4, NaN3 and protease inhibitors
- Filters 0.2 m
- CHAPS, KCl, PBS (PH: 7.2)
- One incubatory shaker (SL) with platforms, flasks and tube adapted accessories
- Histology materials and consumables, tips, glassware, tissue culture plates, etc.
- PCs and software
- Mosquito rearing materials and consumables
- Experimental animals (mice and rabbits)

B) Human Resources:

Two full-time postgrads (MSc and PhD) students will be involved in the project.

Two full-time technicians will be involved in the project.

C) Transportation and Travel or Training:

- Several field trips (including tickets and accommodation) for sampling the target bacteria (from different localities) and mosquitoes (from Riyadh and Eastern region) will be arranged during the course of the project.
- Travel of researcher(s) to and from Saudi Arabia may be needed.
- Convening of two meetings per month for following up on work and results.
- Each member of the team will travel at least once during the project period to attend a conference outside the country.

6.3. PROPOSED BUDGET

SEE INSTRUCTIONS *(in Saudi Riyals)*

PROJECT TITLE		Native mosquito larvicidal bacteria as new candidates in the control of mosquito-borne diseases in Saudi Arabia						
DURATION		(24) MONTHS						
ITEM	CATEGORY	NO.	COMPENSATION	FIRST YEAR		SECOND YEAR		TOTAL
				MONTHS	BUDGET	MONTHS	BUDGET	
MANPOWER	CONSULTANTS	2	2000	5	20,000	5	20,000	40,000
	PRINCIPAL INVESTIGATOR	1	6000	12	72,000	12	72,000	144,000
	CO-INVESTIGATOR	2	5000	10	100,000	10	100,000	200,000
	OTHER SENIOR PERSONNEL							
	POSTDOCTORAL	-	-	-	-	-	-	-
	RESEARCH ASSTISTATS :-	2	2000	10	40,000	5	20,000	60,000
	PHD STUDENTS MS STUDENTS	2	1000	10	20,000	10	20,000	40,000
	UNDERGRADUATE	-	-	-	-	-	-	-
	PROJECT MANAG.	-	-	-	-	-	-	-
	TECHNICIANS	2	2000	10	40,000	10	40,000	80,000
	SECRETARIAL-CLERICAL	1	1000	10	10,000	10	10,000	20,000
	OTHER (worker/driver)	1	1000	6	6000	6	6000	12,000
SUMMER COMPENS.	COMPENSATION 1	-	-	-	-	-	-	-
	COMPENSATION 2	-	-	-	-	-	-	-
TOTAL SALARIES (INCLUDING SUMMER COMPENSATION)				308,000		288,000		596,000
EQUIP. & MATERIAL	MAJOR EQUIPMENTS (> = 100.000)			343,000		-		343,000
	EQUIPMENTS (< 100,000)			50,000		40,000		90,000
	MATERIALS & SUPPLIES			307,000		0		400,000
ITEM TOTAL				600,000		140,000		740,000
TRAVEL	CONFERENCES			25,000		30,000		55,000
	TRAINING			-		-		-
	FIELD TRIPS			100,000		0		100,000
	TICKETS			5,000		5000		10,000
ITEM TOTAL				130,000		35,000		165,000
OTHERS	PATENT REGISTRATION			-		10,000		10,000
	PUBLICATIONS			15,000		15,000		30,000
	WORKSHOP			-		-		-
	OTHER EXPENSES							
ITEM TOTAL				15,000		25,000		40,000
GRAND TOTAL				1027000		462,000		1,541,000

SALARIES (INCLUDING SUMMER COMPENSATION)	%	38.67
EQUIPMENTS & MATERIALS	%	48.02
TRAVEL	%	10.70
OTHERS	%	2.60
GRAND TOTAL	%	100

FORM RE- D1-7

6.4. BUDGET JUSTIFICATION:

The present proposal budget will be distributed between salaries, instruments, chemicals, reagents, experimental animals, DNA extraction kits, travel and conference attendance and running costs.

Itemization	Year 1	Year 2
Salaries		
PI. Dr Ashraf M. Ahmed	72,000	72,000
COI-1. Prof. Talat A. El-Kersh	50,000	50,000
COI-2. Dr Tahany H. Ayaad	50,000	50,000
Consultant-1: Prof Hamdy I. Hussein	14,000	40,000
Consultant-2: Dr Hanan El-Sadaway	32,000	20,000
Research Assistants (2)	40,000	20,000
Technicians (1)	40,000	20,000
Postgrad students (2)	20,000	20,000
Secretarial (1)	10,000	10,000
Other (workers/drivers) (1)	6,000	6,000
Total	**314,000**	**282,000**
Materials and supplies • DNA Extraction kits and Plasmid DNA Extraction Kits • Universal and specific Primers • SDS/PAGE materials for proteins and DNA cry genes agarose gel separation (electrophoresis) • Gel accessories track dyes molecular wt standards, buffers • Culture media, yeast extract, LB, BHI, TSA, with additives, Packed RBCs • Antibiotic discs for susceptibility patterns of *B thurengiensis* • Glassware EM flasks, 125, 250, 500, and 1000 mlcapacity • RPMI 1640 culture media • Fetal calf serum • API CH 50 kits, Gram(+) Bacilli-identification • Supplementary API 20 kits • CCl4, acetone, solvents, phenol, CHCl3 • Na2SO4, NaBr, Poly E G 6000, Sucrose • Cesium chloride for Crystal/spore separations		

• Osmium Oxide, Glutaraldehyde, Buffers for SEM of crystals • Sera Kits for *B. thurengiensis* serotyping • Gloves, disposable pages • Glycerol, Lactose • Sonicator for crystal/spore separation • Protease Peptone no. 3 (PP3), Nutrient broth • Nutrient Agar, Lb media and Muller Hinton Agar and Blood agar base, MHA media • Polyoxyethylene sorbitan monostearate (Tween 80, 60, 40, and 20) • srDNA kits and 18 srDNA kits • Glycerol binuclear • Nuclear free water • Polyacrylamide gel electrophoresis and relevant chemicals • Dialysis bags low molecular weight • Protein markers kits • mM Tris-HCl pH 8.0 and 1 mM DTT (dithiothreitol) • Glycerol • Lysozyme and Bacterial protease inhibitor cocktail • Q Sepharose XL (1.6 × 10 cm) column, Superose 200 size (1.6 × 60 cm) column, MonoQ (0.5 × 5 cm) column, N-terminal amino acid, Superdex 200 10/30 gel filtration column, dextran/gold CM-5/ CM-4 Biacore chip, 3-dimethylaminopropyl, 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC), Ethanolamine and N-hydroxysuccinimide (NHS) • HPS-EP buffer (BiaCore), EDTA, MgSO4, NaN3 and protease inhibitors • Filter 0.2m • CHAPS, KCl, PBS (PH: 7.2) • One shaking incubator (SL) with platforms, flasks and tube adapted accessories • Histology materials and consumables, tips, glassware, tissue culture plates, etc. • PCs and software • Mosquito rearing materials and consumables • Experimental animals (mice and rabbits)		
Total	**307,000**	**0**

Instruments	300,000	40,000
Total:	**393,000**	**40,000**
Travel:		
Conferences	25,000	30,000
Field Trips	100,000	--
Tickets	5,000	5,000
Total:	**130,000**	**35,000**
Others		
Patent Registration	--	10,000
Publication	15,000	15,000
Other expenses (maintenance, replacements, office consumables …etc)	--	- -
Total:	**15,000**	**25,000**
TOTAL BUDGET	1,159,000	382,000
NET BUDGET 1,541,000		

7. UNDERTAKING OF THE RESEARCH TEAM:

The research team undertakes that:

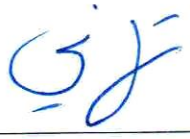
1- **The text and graphics herein as well as any accompanying publications or other documents, unless otherwise indicated, are the original work of the signatories or individuals working under their supervision.**

2- **No part of this proposal has been funded by any other source.**

3- **No existing funds are available to the research being proposed from any other source.**

4- **No fund would be sought from any other source if an award is made as a result of this proposal.**

5- **We agree to accept responsibility for the scientific conduct of this project.**

ROLE	INVESTIGATOR NAME	SIGNATURE
Principal Investigator (PI)	**Dr. Ashraf Mohamed Ahmed Ali**	
CO- PI. 1	Prof. Talat Abd Monsef El-Kersh	
CO- PI. 2	Dr Tahany Hassan Ayaad	
CO- PI. 3		
CO- PI. 4		
CO- PI. 5		

Form RE- D1-8

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9. RESUMES

CURRICULUM VITAE-1 (PI)

DR Ashraf M. Ahmed

PERSONAL DETAILS

Name:	Ashraf Mohamed Ahmed
Nationality:	Egyptian
Date of birth:	9 / 10 / 1967
Marital status:	Married (3 children)
Address:	Zoology Department, Faculty of Sciences, El-Minia University, El-Minia, Egypt.
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ACADEMIC QUALIFICATIONS

Bachelor of science: (Entomology**)**	Entomology Department, University of Zagazeeg, Benha Branch, Egypt (May, 1990).
MSc in Entomology; (Insect Immunity**):**	Zoology Department, University of El-Minia, Egypt (March, 1993- July, 1995). **Title:** Some immune response mechanisms of the cotton leaf worm *Spodoptera littoralis* and silk worm *Bombyx mori* to some biological and non-biological agents.
PhD in Entomology; (Vector Biology & Immunity**):**	Biological Sciences Department, Keele University, UK (January, 1998 - January, 2002). **Title:** Molecular approaches to the effect of malaria infection on anopheline mosquito reproductive fitness.

TEACHING EXPERIENCES

Has teaching experiences in Entomology and Zoology in both Saudi Arabia and Egypt

RECENT TEACHING INTERESTS

General Entomology, Medical Entomology, General Animal Biology, Animal Ecology and others

RESEARCH INTERESTS

- **Insect Immunity and Physiology:** Studying the different immune responses of insects aiming to utilizing the innate immune system in enhancing the beneficial insect and controlling the harmful one.

1. ***Plasmodium*-Mosquito interaction:** Studying the possibility of utilizing the innate immune system of mosquito vector to control malaria parasite.

- **Insect Biological Control:** Investigating new natural microorganisms suitable for use as biological control agents against mosquito vectors.

POSTGRADUATE SUPERVISIONS

I)- MSc thesis:

2. One currently ongoing MSc, studying the possibility of utilizing some native entomopathogenic bacteria for controlling the Egyptian cotton-leaf worm in El-Minia Governorate (Zoology Department, El-Minia University).
3. One currently ongoing MSc, studying the possibility of utilizing some native entomopathogenic fungi for controlling the Egyptian cotton-leaf worm in El-Minia Governorate (in collaboration with Botany Department, El-Minia University).

4. One currently ongoing MSc, utelizing the immune responses of honey bee, *Apis florae* against bee-threatening *P. larvae* bacteria.
5. One currently ongoing MSc, studying the immune responses of the poisonous samsum ant *P. sennaarensis*.
6. One successfully completed MSc on ecological survey of the poisonous samsum ant *P. sennaarensis* in Ehsaa' Region in Saudi Arabia.
7. One successfully completed MSc on biocontrol of *Ae. caspius* and *Cx. pepiense* mosquitoes using a native mosquitocidal *Pseudomonas frederiksbergiens* bacterium.

II)- PhD thesis:

- One successfully completed PhD on molecular effect of *B. thurengiensis* on the mosquito vector, *Ae. caspius*.
- One successfully completed PhD on Ecological survey of the poisonous samsum ant *P. sennaarensis* in Riyadh Region.

PARTICIPATION AT MEETINGS

A)- In Egypt:

1)- The 1st Congress of Sciences and Development:
Organised by the Faculty of Science, El-Azhar University, EGYPT, 20th –23rd of March, 1995.

2)- The 3rd Congress of Toxicology In Developing Countries:
Organised by the National Research Centre, Cairo, EGYPT, 19th –23rd of November, 1995.

3)- The 3rd International Conference on Biological Sciences:
Organised by University of Tanta, Tanta, EGYPT, 28th – 29th of April, 2004.

4)- The 1st International Conference on Natural Toxins:
Organised by Faculty of Pharmacy, October 6 University, Cairo, EGYPT, from 18th – 19th of December 2004**.**

5)- The 15th International Conference of the Egyptian German Society of Zoology:
Organized by Faculty of Girls, Ain Shams University Cairo, EGYPT, from 26th of February – 2nd of March 2005.

6)- The 3rd International Conference of Applied Entomology:
Organized by Department of Entomology, Faculty of Sciences, University of Cairo, EGYPT, from 23rd – 24th of March 2005.

B)- In Britain & International:

1)- The 10th Malaria Meeting:
Organised by the British Society for Parasitology (BSP), September, 21–23, 1998, at the University of Edinburgh, Edinburgh, UK.

2)- The 11th Malaria Meeting:
Organised by the British Society for Parasitology (BSP), 20th – 22nd of December, 1999, at Imperial Collage, London, UK.

3)- The XXXI International Congress of Entomology:

20th – 26th of August, 2000, in Iguassu Falls, Brazil.

4)- Research In Progress (Short Presentations & Posters)

Organised by The Royal Society of Tropical Medicine and Hygiene, 7th of December, 2000, at Manson House, London, UK.

5)- Joint Malaria and Spring Meeting

Organised by British Society for Parasitology (BSP), 17th – 20th of April, 2001, at Keele University, Stock-On-Trent, UK.

6)- Workshop on Ecological Immunity of Arthropods

Organised by the European Society Foundation, 6th – 9th of December, 2001, at Losehill Hall, Sheffield, UK).

7)- Joint Malaria and Spring Meeting:

Organized by the British Society for Parasitology (BSP) at the University of Nottingham, Nottingham, UK, from 3rd - 6th of April, 2005.

8)- XI International Congress of Parasitology (ICOPA XI):

Organized by the British Society for Parasitology (BSP) at Scottish Exhibition & Conference Centre (SECC), Glasgow, Scotland from 6-11 August 2006.

9)- NACON VIII: 8th International Meeting on Recognition Studies in Nucleic Acids

Organized by Biochemistry Department, Sheffield University on12-16th September, 2010, UK.

10)- BSP Annual Spring Meeting, Nottingham, UK,

Organized by British Society for Parasitology, Monday 11th April to Thursday 14th April 2011.

C)- In Saudi Arabia

The 3rd Saudi Conference:

Organized by the College of Science, King Saud University KSA from 10-13th of March 2007.

PROJECTS & PERSONAL ACTIVITIES

A)- Funded Research Projects:

1. The Use of Insect Venoms as Major New Tools in the Fight against Breast Cancer in Saudi Arabia. Funded by King Saud University (ongoing; Budget: One million US Dollar, from 2009-2012).
2. Development and Production of Molecular Identification kits for Monitoring of Mosquitoes Vectors in Open Fields. Funded by The Excellent Centre for Biotechnology Research, King Saud University (ongoing, Budget: Nine Hundred thousand Saudi Rials, from 2009-2011).
3. Ecological Survey of Samsum ant in Riyadh Region. Funded by King Saud University (completed successfully, Five Hundred Thousand Saudi Riyals, from 2008-2010).
4. Enhancing the Humoral and Melanization Responses of *Aedes aegypti* Mosquito: a step towards the utilization of immune system against dengue fever. Funded by the research Centre, Faculty of Science, King Saud University (completed successfully, Budget: Fifty Thousand Saudy Rials, from 2007-2008).

5. The Immune Enhancer, Thymoquinone, and the Hope of Utilizing The Immune System of *Aedes caspius* Against Disease Agents. Funded by the research Centre, Faculty of Science, King Saud University (completed successfully, Budget: Thirty Five Thousand Saudy Rials, from 2008-2009).
6. Studying Trail Pheromone and Immune Responses *Monomorium* ants: A step towards integrated control. Funded by the research Centre, Faculty of Science, King Saud University (completed successfully, Budget: Forty Five Thousand Saudy Rials, from 2009-2010).
7. There are some other currently prepared projects ready to be submitted to different funding sources.

LIST OF PUBLICATIONS

1. **Ahmed, A. M**.; Mahmoud, A. A. and Al-Qahtani, H. M. (2011). Utilization of a Novel Bacterial Extract Against Types of Mosquito Vectors larvae in Saudi Arabia: a promising environmentally safe bioinsecticide. (In Preparation).
2. **Ahmed, A. M.**, Shaalan, E. A., Aboul-Soud, M. A. M. and Al-Khedhairy, A. A., Mosquito vectors survey in AL-Ahsaa district, eastern region, Kingdom of Saudi Arabia. Journal of Insect Science (In Press).
3. Mashaly, A. M. A.; **Ahmed, A. M**.; Al-Abdullah, M. A.; AI-Khalifa, M. S.; Siddiqui, M I. (2011). The trail pheromone of the venomous samsum ant, *Pachycondyla sennaarensis*. Journal of Insect Science, 11: 1-12.
4. Al-Roba1, A. A., Aboul-Soud, M. A. M., **Ahmed, A. M.** and Al-Khedhairy, A. A. (2011). The gene expression of caspasses is up-regulated during the signaling response of *Aedes caspius* against larvicidal bacteria. African Journal of Biotechnology. 10 (2): 225-233.
5. Sediqi, M. I.; Mashaly, A. M. A.; **Ahmed, A. M.** and Al-Khalifa, M. S. (2010). Ultrastructure of Antennal Sensilla of the Samsum ant, *Pachycondylla sennaarensis* (Hymenoptera: Formicidae: Ponerinae) from Saudi Arabia. African journal of biotochnology (In press).
6. Mashaly, A. M. A.; **Ahmed, A. M**.; Al-Khalifa, M. S.; Nunes, T. M. and Morgan, E. D. (2010) Identification of the alkaloidal venoms of some *Monomorium* ants of Saudi Arabia. Biochemical Systematics and Ecology, 38: 875–879.
7. Al-Khalifa, M. S.; **Ahmed, A. M**.; Mashaly, A. M. A.; Al-Mekhalfi, F. A.; Khalil, G.; Siddiqui, M. I. and Ali, M. F. (2010). Studies on the Distribution of *Pachycondyla sennaarensis* (Hymenoptera: Formicidae: Ponerinae) in Saudi Arabia. 1. Ar-Riyadh Region. Pakistan J. Zool., vol. 42(6): 707-713.
8. **Ahmed, A. M**., Al-Olayan, E. M. Aboul-Soud, M. A. M. and Al- Khedhairy, A. A. (2010). The immune enhancer, thymoquinone, and the hope of utilizing the immune system of *Aedes caspeus* against disease agents. African Journal of Biotechnology, 9(21) : 3183-3195.
9. **Ahmed, A. M**., Al-Olayan, E. M. and Amoudy, M. A. (2008). Enhancing the humoral and melanization responses of *Aedes aegypti* mosquito: a step towards the utilization of immune system against dengue fever. Journal of Entomology. 5(*5*): 305-321.
10. **Ahmed, A. M.** and El-Katatny, M. H. (2007). Entomopathogenic fungi as biopesticides against the Egyptian cotton leaf worm, *Spodoptera littoralis*: between biocontrol-promise and immune-limitation. *Journal of Egyptian Society of Toxicology*. **37:** 39-51.

11. **Ahmed, A. M.** and Hurd, H. (2006)**.** Immune stimulation and malaria infection impose reproductive costs in *Anopheles gambiae via* follicular apoptosis. *Microbes and Infection*, **8**: 308–315.
12. **Ahmed, A. M.** (2007). A Dual Effect for the Black Seed Oil on the Malaria Vector A*nopheles gambiae*: Enhances Immunity and Reduces the Concomitant Reproductive Cost. *Journal of Entomology*, **4(1):** 1-19.
13. **Ahmed, A. M.** (2005)**.** The humoral anti-bacterial response of *Anopheles gambiae* and the immunity-reproduction trade-off conflict: between the hope and limitation of the malaria immuno-control strategy. *Proceedings of The 3rd International Conference of Applied Entomology*, Cairo University, 23rd – 24th of March (2005), 351-374.
14. **Ahmed, A. M.** (2005). Melanization of Sephadex beads by the malaria vector, *Anopheles gambiae*: effect of blood meal, and mechanisms of reproductive costs. *The Egyptian German Society of Zoology*. **47(E)**: 69-85.
15. **Ahmed, A. M.** (2004). Activation of the immune system of *Anopheles gambiae* against malaria parasite: a comparison between bacterial infection and a botanical extract. T*he 3rd International Conference on Biological Science. University of Tanta, Tanta, EGYPT, 28 – 29 April. Proc. I.C.B.S.*, **3(1)**: 122 - 141
16. **Ahmed, A. M., S.** Baggott, R. Maingon and H. Hurd (2002). The costs of mounting an immune response are reflected in the reproductive fitness of the mosquito, *Anopheles gambiae. OIKOS* **97:** 371–377.
17. Hopwood, J. A., **Ahmed, A. M**., Polwart, A., Williams, G. T. and Hurd, H. (2001). Malaria-induced apoptosis in mosquito ovaries: a mechanism to control vector egg production. *The Journal of Experimental Biology*. **204:** 2773-2780.
18. **Ahmed, A. M.**, Rhayza Maingon, Patricia Romans and Hilary Hurd (2001). Effects of malaria infection on vitellogenesis in *Anopheles gambiae* during tow gonotrophic cycles. *Insect Molecular Biology*. **10(4)**: 347-356.
19. **Ahmed, A. M.**, R. D. Maingon, Taylor, P. J. and H. Hurd (1999). The effect of infection with *Plasmodium yoelii nigeriensis* on the reproductive fitness of the mosquito *Anopheles gambiae. Invertebrate Reproduction and Development*. **36**: 217-222.
20. Abu El-magd, A. A., Hamed, M. S., El-Kifl, T. A. and **Ahmed, A. M**. (1994). *In vitro* studies on cellular and humoral reactions of *Spodoptera littoralis* larvae to *Bacillus thurengiensis* bacteria and spore-δ-endotoxins. *Bulletin of Faculty of Science, Assute University.* **23(2-E)**: 201-214.

With my complements ***Ashraf M. Ahmed; July, 2011***

CURRICULUM VITAE-2 (COI-1)

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Nationality : Egyptian, Canadian
Languages : English, French & Arabic

DEGREES

1. B.Sc. Pharm. (Honn), Cairo University, Egypt, 1968.
2. M.Sc. Pharm (Microbiology), Cairo University, 1972.
3. Ph.D. Pharm. (Microbiology), Montreal University, Canada, 1975.
4. Higher Diploma (Biochemistry Analysts), Cairo University, Egypt, 1983.

SCHOLARSHIPS & AWARDS

- 1973 – 1976: Studentship (Ph.D Studies), Le Conseil de la Recherch en Sante du Quebec, held Fact. Pharm,Montreal University, Canada.
- 1976 – 1978: Post-Doctoral Fellowship, Medical Research Council of Canada (MRC), held at Dept. of Microbiology & Immunology Faculty of Medicine, Montreal University, Canada.

- 1978 – 1979: Post-Doctoral Industrial Fellowship, National Research Council of Canada (MRC), held at Department of Microbiology, Ayerst Research Laboratories, Montreal , Canada.

POSITIONS HELD

1- 1979 – 1985: Lecturer of Microbiology , Cairo University, Cairo, Egypt.
2- 1985 – 1990: Associate Professor of Microbiology, Cairo University, Cairo, Egypt.
3- 1990 – 1992: Professor of Microbiology and Chairman of Department of Microbiology & Immunology, Faculty of Pharmacy, Cairo University, Cairo, Egypt.
4- 1992 – Date (2010): Professor of Microbiology, Department of Clinical Laboratory Sciences, College of Applied Medical Sciences, King Saud University, Saudi Arabia.

TEACHING CAPABILITIES

- Basic Microbiology , Biochemistry & Physiology.
- Clinical Bacteriology.
- Immunology , Epidemiology and Medical Virology.
- Medical Mycology and Parasitology.
- Antimicrobial Agents and Susceptibility Testing.
- Medical Terminology and General Pathology.
- Safety, Quality Control and Laboratory Management.
- Clinical Specimens and Microbiology Practice

COMMITTEE MEMBERS

1. Co-ordinator of the Academic Advisor committee for BSc students CLS,CAMS,KSU,sa
2. Member of the Saudi Society of Clinical Laboratory Sciences &Medical Specialties, SA
1- Regular Reviewer for the committee of Medical classification at the Saudi Commission for medical specialist
3. Member of the committee of RESEARCH centre CAMS,KSU,sa
4. Member of the Academic committee of BSc curriculum study plan CLS dept. CAMS, KSU, SA.
5. Regular Reviewer of KACST grand projects and final reports

List of <u>PUBLICATIONS OF PROF. TALAT EL-KERSH</u>

8. TOAMA, M.A.; T.A. EL-KERSH, and M. ABDEL-AZIZ, 1970. Screening for cellulolytic fungi from Egypt, XII Conference of Pharm. Sci., November 22 – 25, 1970, Cairo, Egypt, P. 70.
9. TOAMA, M.A.; T.A. EL-KERSH, and M. ABDEL-AZIZ, 1970. Optimum conditions for cellulose production by *Phoma sp*. And *Rhizoctonia sp*. [bid P. 71].
10. TOAMA, M.A.; T.A. EL-KERSH and M. ABDEL-AZIZ, 1970. Characterization of cellulase enzyme from *Phoma sp*. [bid P. 72].
11. EL-KERSH, T.A.; M.A. TOAMA, and M. ABDEL-AZIZ, 1973. Cellulase production by *Phoma glomerata and Rhizoctonia solani.*. I. Optimal conditions for the production of the enzyme Chimie Microbiologie Technologie Alimentaire 2:102-106.
12. EL-KERSH, T.A.; M.A. TOAMA, and M. ABDEL-AZIZ, 1975. Cellulase production by

Phoma glomerata and Rhizoctonia solani. II. Characterization of Phoma-cellulase, Chem. Mikrobiol. Technol. Lebensin 4:58-64.
13. EL-KERSH, T.A.; and J.R. PLOURDE, 1973. Microbial transformation of antibiotics, Research Symposium of the A. F. P., Dalhousie University, Halifax, May 4-5, 1973.
14. EL-KERSH,T.A., and J.R.POURDE,1974..Microbial transformation of antibiotics.I. Mechanism of chloramphenicol inactivation by the spores of *Streptomyces* sp. Isolated from soil. 21st Canadian Conf. on Pharmaceutical Research, Morisset Hall University of Ottawa, May 20, 1974.
15. EL-KERSH, T.A., and J.R. PLOURDE, 1974. Microbial transformation of antibiotics II. Isolation and characterization of a *Streptomyces s*p. Capable of inactivating chloramphenicol, Clindamycin and kanamycin and showing antibacterial activity, [bid, 1974].
16. PLOURDE, J.R., and T.A. EL-KERSH, 1974. Transformation microbienne des antibiotiques, III. Transformation de la griscofulvine par *Rhizopus nigricans* et *Penicillium thomii*, Annales de PACFAS, Universite Laval, Laval, Qeubec, 11:150, No. 1, 1974.
17. EL-KERSH, T.A., and J.R. PLOURDE, 1975. Microbial transformation of antibiotics IV. Some properties of chloramphenicol acetyltransferase in a *Streptomyces sp*. Isolated from the soil, 22nd Canadian Conference on Pharmaceutical Research, Universite de Montreal, Montreal, May 14-16, 1975.
18. EL-KERSH, T.A., and J.R. PLOURDE, 1975. Microbial transformation of antibiotics V. Localization of chloramphenicol acetyltransferase in the spores of a *Streptomyces sp*. [bid, 1975].
19. EL-KERSH, T.A., and J.R. PLOURDE, 1976. Microbial transformation of antibiotics, VI. Specificity of chloramphenicol acetyltransferase (CAT) in the spores of *Streptomyces griseus* in Proceedings of the 19th Annual Meeting of Canadian Federation of Biological Societies, 15-18 June, Dalhousie University, Halifax, 19:528.
20. EL-KERSH, T.A., and J.R. PLOURDE, 1976. Biotranformation of antibiotics, I. Aceylation of chloramphenicol by spores *of Streptomycis griseus* isolated from the Egyptian soil J. Antibiotics 29.292-302.
21. EL-KERSH, T.A., and C. VEZINA, 1978. Plasmid determination of antimycin A production in auxotrophs of *Streptomyces sp*. M-506. Third International Symposium on the Genetics of Industrial Microorganisms, June 4-9, 1978. Madison, Wisconsin, Paper 10.
22. EL-KERSH, T.A., and C. VEZINA, 1978. Effect of plasmid-curing agents on antibiotic productivity of steptomycetes, Annual meeting of the Canadian Society of Microbiologists, June 11.15, 1978, Montreal, Que., Paper A6.
23. EL-KERSH, T.A., and J.R. PLOURDE, 1980. Biotransformation of antibiotics III. Localization of chloramphenicol acetyltransferase in spores of *Streptomyces griseus* and specific formation of chloramphenicol 3-acetate by this enzyme, European J. Appl. Microbiol. Biotechnol. 10 317-326 (1980).
24. EL-KERSH, T.A., and C. VEZINA C, Localization of antimycin A and melanin determinants in auxotrophs *Steptomyces sp*. M-506 and in their protothropic revertants. Proc. 6th Intern. Fermentation Symposium, London, Canada, July 20-25, 1980. In Advances in Biotechnology Vol. III, eds., Claude Vezina & Kartar Singh. P. 37-42, Pergamon Press N.Y. (1980).
25. TOAMA, M.A., EL-KERSH, T.A. and AHMADY A.M. Antibiotic-resistant pattern and resistance curing of *Escherichia coli* locally isolated. Bull. Fac. Pharm., Cairo Univer. Vol. XXII No. (1) P. 1-19 (1983).

26. TOAMA, M.A, EL-KERSH, T.A. and M.A. RAMADAN. Inhibition of aflatoxin release by selected insecticides and benzoie acid derivaties. In Intern. Mycotoxin Conf. I. Cairo, Egypt, March 19-24, 1983 sponsored by NRC Cairo, Egypt & FDA Washington DC, USA, Paper 17 (1983),
27. TOAMA, M.A. EL-KERSH, T.A. and M.A. RAMADAN. Multi-mycotoxin detection in Egyptian herbal drugs. In Intern. Mycotoxin Conf. I. Cairo, Egypt, March 19-24, 1983 spondored by NRC Cairo, Egypt & FDA Washington DC, USA, Paper 18. (1983).
28. TOAMA, M.A., EL-KERSH, T.A. and M.A. RAMADAN. Mycotoxin-producing fungi isolated from Egyptian Herbal Drugs, Proc. 5th Conf. Microbiol., Cairo, Egypt, May 21-23, 1983.
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34. EL-KERSH, T.A., EL-ZAWAHRY, Y.A., ABU EL-KHEIR, A. and ABD EL-LATIF, H.K. (1985). Radiation and thermal resistant of bacteria isolated from some raw materials. The first International Conference of Applied Sciences, Zagazig University, Zagazig, Egypt, 30 March, 1 Arpil, Vol. II.p. 201, (1985).
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Agents-Resistance and Colicin Production. Mansoura Journal of Pharm. Sciences. Accepted for publication.
40. EL-KERSH, T.A., EL-SAYED, M.A., and EL-MASSRY, E.M., (1986). Studies on Locally Isolated Enteropathogenic E. Coli. II. Intrageneric transfer of R. Plasmid(s) conferring resistance to antibiotics. Egypt.J. Appl. Sci., 1,(i), 91-96.
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48. - El-Tayeb, O.M., El-Kersh, T.A., Hashem, A.M., and Radwan, H.H.(1989). Microbial mycolytic activity. II. Factors affecting mycolases productivity of Streptomyces rimosus. Bull. Fac. Pharm. Cairo Univ. 27, (2), 8-12.
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51. - El-Tayeb, O.M., El-Kersh, T.A., Hashem, A.M., and Radawn, H.H.(1989). Microbial mycolytic activity. V. Selection of Streptomyces rimosus (S21) variants with enhanced chitinase and mannanase production. Bull. Fac. Pharm. Cairo Uni. 27, (2), 23-26.
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53. MONA, A. EL-SAYED. T.A. EL-KERSH, F.F., SERRY and HEMMAT, A. LATIF (1990). Microbial cyanocobalamine productivity II. Streptomyces termietum and Bacillus freudorcichii strain improvement. AZ J. Microbiol. 9, (93-105).
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56. MONA A EL-SAYED; EL-KERSH, TA; EL-BEHARRY,AH. & OKASHA, MM.(1990). Investigation of chronic bacterial prostatitis among Egyptian patients 11.Diffusion and protein binding of clindamycin , erythromycin , lincomycin and trimethoprim . AZ.J .Microbiol.,10,(1-14)
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60. EL-KERSH ,TA ,El-Sayed, MA,and El-Masry EM.(1992) Genetic Manipulation for High Cellulase Productivity by Streotomyces roseoflavus. Zag.Vet.J.vol20(6):pp1057-1073.
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62. EL-KERSH, TA;TAWFIK, AF; AL-SHAMMARY, F; AL-SALEH ,S; KAMBAL, AM; and SHIBL, AM.(1995) .Antimicrobial resistance and prevalence of extended spectrum B-lactamase among clinical isolates of Gram-negative bacteria in Riyadh . J. Chemotherapy, 7: 509-514.
63. AL-ZAMEL, FA; TAWFIK, AF; AL-SHAMMARY, FJ; EL-KERSH, TA; KAMBAL, AM; and SHIBL, AM.(1996).Antibiotic resistance and serotypes of clinical isolates *Pseudomonas aeruginosa* in Riyadh. Med. Sci. Res. ,24: 103-105.
64. AL-REFEA ,AO; AL-ZAMEL , FA; EL-KERSH , AT ; and MOSTAFA FM. (1998). Antibiotic resistance and B-lactamases detection among enterobacteriaceae isolated from Madinah hospitals.Saudi Pharm. J. ,6 : 151-156.
65. AL-REFEA ,AO ; EL-KERSH , TA ; and MOSTAFA , FM. (1998). Antibiotic resistance and serotypes of *Pseudomonas aeruginosa* isolates from Madinah hospitals . Saudi Pharm. J. 6:146-150.
66. AL-AWAJI , A ; MOSTAFA , FM ; EL-KERSH , TA ; and AL-SHAMMARY , FJ.(2000). Susceptibility of enterococcal isolates from Riyadh city hospitals to some antimicrobial agents .Saudi Pharm. J. ,8 : 43-50.
67. AL-AWAJI ,A ; MOSTAFA , FM ; EL-KERSH , TA ; and AL-SHAMMARY , FJ .(2000). Response of high level gentamicin resistant enterococci to penicillin and gentamicin combination .Saudi Pharm. J. ,8 : 51-57.
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antibiotics. Bull. Pharm. Sci., ASSIUT University, 27: 133-144.
71. EL-KERSH , T.A ; NASSER , L.A ; and MEJALY , SH (2009) Virulence factors of Group B Streptococci isolated from dairy products and clinical specimens in Riyadh region .Saudi Med. J ., submit
72. MATTAR EH *, HAMMAD LF *, AHMAD S**, EL-KERSH T A*** An Investigation of the Bacterial Contamination of Ultrasound Equipments at a University Hospital in Saudi Arabia. JOURNAL OF CLINICAL AND DIAGNOSTIC RESEARCHJCDR doi:611-1013-1024(published online first 19th May 2010)

CURRICULUM VITAE-3 (COI-2)

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ACADEMIC BACKGROUND

DEGREE Ph.D.
Major Insect Physiology (Immunology)
Institution Faculty of Science,Cairo University, Egypt
Date Granted April, 1996

DEGREE M.Sc**.**
Major Insect Physiology (Immunology)
Institution Faculty of Science, Cairo University, Egypt
Date Granted May, 1988

Degree B.Sc.
Major Entomology
Grade Distinction
Institution Faculty of Science, Cairo University, Egypt
Date Granted June 1981

M. Sc. In the field of Insect Physiology &Immunology. Cairo University. Thesis entitled "Cellular and humoral immunity of the cotton leafworm *Spodoptera littoralis".*

Ph. D. In the field of Insect Physiology &Immunology. Cairo University. Thesis entitled "Immunological studies on *Spodoptera littoralis* Boisd. (Lepidoptera, Noctuidae) and *Parasarcophaga dux* Thomson (Diptera, Sarcophagidae)".

Academic Experience

- Demonstrator in the Department of Entomology , Faculty of Science ,Cairo University. 10/1981.
- Assistant lecturer in the Department of Entomology , Faculty of Science ,Cairo University. 5/1988.
- Lecturer in the Department of Entomology , Faculty of Science , Cairo University. 4/1996.
- Associate Professor in the Department of Entomology , Faculty of Science , Cairo University. 30/6/2004.
- Professor of Insect Physiology & Immunology in the Department of Entomology , Faculty of Science , Cairo University, Egypt (27/1/2010) .
- Associate Professor in the Department of Zoology , Faculty of Science , King Saud University. (14/9/2006) up till now.

Teaching Experience

- Lecture courses and Practical courses to the undergraduate and postgraduate students in the Department of Entomology , Faculty of Science , Cairo University (1981 -2006).
- Lecture courses and Practical courses to the undergraduate and postgraduate students in the Department of Zoology , Faculty of Science ,King Saud University **(2006 uptill now).**

Theses Supervision

Ph. D. Thesis.

1- Susceptibility of *Culex* (*culex) pipiens* L. (Diptera: Culicidae) to the infection with *Hepatozoon gracilis* (Apicomplexa: Hepatozoidae) of the Egyptian bean skink *Mabuya quinquetaenita quinquetaenita* (2005). Granted.

2- Interactions of entomopathogenic nematodes with the effects of pharmaceutical inhibitors of eicosanoid biosynthesis on the desert locust *Sc.gregaria* fifth instar nymphs (2006). Department of Entomology , Faculty of Science, Cairo University , Egypt . Granted.

3- Inter and Intra Specific Variability of Some Sandfly Species in the Most Prevalent Regions of Saudi Arabia Based on Nucleotide Sequences of Internal Transcribed Spacer (ITS) of the Gene Coding for rRNA. King Saud University, Faculty of Science, Department of Zoology. Under investigation.

Master Thesis.

- Study on the effects of soft ticks (Acari: Argasidae) on chicken and using entomopathogenic nematodes in biological control of this ectoparasite (2002). Ain Shams University, Faculty of Women, Department of Zoology. Granted.

- The immune response of the Syrian hamster antibodies to mosquitoes midgut extracts and its effects on the biological and physiological activities of *Aedes aegypti* ,the Dengue fever vector. King Saud University, Faculty of Science, Department of Zoology. Granted.
- Efficacy of the Entomopathogenic Nematodes Towards the Red Palm Weevil *Rhynchophorus ferrugineus* (Olivier) Larvae Using Inhibitors of the Insect Cellular Immune Response. King Saud University, Faculty of Science, Department of Zoology. Granted.
- Study of the Honey Properties and Isolation of Antimicrobial Peptides of Wild and Carinolian Honeybees in the Central Region of Saudi Arabia. King Saud University, Faculty of Science, Department of Zoology. Granted.
- Using Entomopathogenic Bacteria-Nematode Symbiotic Toxins as an Insecticide on *Culex quinquefasciatus* Mosquitoes. King Saud University, Faculty of Science, Department of Zoology. Granted.
- Prevalence and Genotypic Analysis of *Toxoplasma gondii* among Saudi Pregnant Women in Riyadh City, Saudi Arabia. King Saud University, Faculty of Science, Department of Zoology. Under Investigation.
- Purification and Characterization of an Immune Lectin Component from Samsum Ant *Pachycondyla sennaarensis* in Saudi Arabia. Hail University , Faculty of Science , Department of Zoology, Saudi Arabia. Under Investigation .

Conferences Participation

1- Membership in the workshop of the Eighth Conference of Egyptian Zoology society (February 2000). Cairo University, Faculty of Science.
2- Membership in the board of the First Efflatoun Conference of Entomology (March 2001). Cairo University. Faculty of Science.
3- Membership in the board of the Second Conference of Applied Entomology. 2002.
4- Membership in the thirteenth conference of the Egyptian German Society (March 2003); (paper).
5- Membership in the workshop of the first international students conference in biotechnology (November 2010) . Faculty of Science , Cairo university , Egypt.

Administrative Activities

1. Membership in constructing the scientific Photography and Microscopic laboratory. Department of Entomology- Faculty of Science, Cairo University.
2. Membership in constructing computer laboratory in Department of Entomology, Faculty of Science , Cairo University.
3. Membership in constructing the molecular biology and immunology laboratory in Department of Entomology ,Faculty of Science ,Cairo University.
4. Membership in constructing the central laboratory in Department of Entomology, Faculty of Science ,Cairo University.
5. Membership in constructing the entomology and parasitology laboratory in the Zoology Department, Faculty of Science , King Saud University.
6. Membership in improving the post graduate courses in the field of Entomology and parasitology Department of Zoology ,Faculty of Science , King Saud University.

7. Membership of the Commission of the Annual Report of the Zoology Department, Faculty of Science , King Saud University.

Membership in the International Projects

5. Membership in Cairo University , Faculty of Agriculture Project "Studies on resistance of different farm animals and poultry to parasitic Acarine infestation and the production of Acarine vaccine". Centre of Acaros research , Faculty of agriculture. Research project 416-B.In the period from 1997-1999. Supported by the Ministry of Economy and International Cooperation (Department of Economic Cooperation with USA).

Professional Societies Participation

- Entomological Society of Egypt
- Egyptian Society of Biochemistry
- Egyptian Society of Parasitology
- Egyptian Society of Zoology
- Egyptian German Society
- Arab Journal of Biotechnology
- Egyptian Society for Biological Control of Pests (ESBCP)

Training Courses and Workshop Participation

- French course "Medium level". Cairo University in cooperation with the culture and educative French center, French Embassy in Egypt. 11/11/1996 – 30/4/1997.
- "Pollution of the Environment with parasitic protozoons" Ophthalmic Research Institute. 1-10/12/1999.
- Modern techniques in Biotechnology. Cairo University, Faculty of Agriculture. Parasitic Acarine Research Center. 16-19/4/2001.
- "DNA -Finger Printing " "VACSERA". 21-24/9/2002
- "The Role of Chemical and Biological Analyses in Environmental and Industrial Management" The Micro Analytical Centre, Faculty of Science, Cairo University. 5-9/12/2004.
- "One day Seminar on Synchrotron Applications" Faculty of Science, Cairo University 27 /7/2008.
- Training Workshop on Immunological in Department of Zoology and Central Lab in King Saud University Medical Studies & Sciences Sections " one day workshop 'hands on' training on immunological techniques covering important areas in immunology". (May 2011). Participation : **Trainer .**

List of Publications

- **Ayaad,T. H.(2009).** Isolation of Midgut Agglutinin of *Culex quinquefasciatus* (Diptera: Culicidae).Egyptian Academic Journal of Biological Science Entomology, A2 (2):55-64.
- **Ayaad, T.H.(2008).** Isolation and partial characterization of Ca^{2+}-independent lectin from *Aedes caspius* (Diptera: Culicidae). Efflatounia, 8 :17 – 28.

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- Arabia and Investigation of the Antimicrobial Properties of Natural Honey Samples.Journal of King Saud University (Sciences) , In press .
- **Ayaad, T. H.** Zohdi, N., Shairra S. A. and Ibrahim A.A. (2008) Effects of Entomopathogenic Nematodes and Some Pharmaceutical Inhibitors of Ecosanoide Biosynthesis on the Desert Locust *Schistocerca gregaria* Frost.The Proceeding of the2nd Arabian Conference for the Biological Control Applications for Pest Control (7-10 April 2008).
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CURRICULUM VITAE-4 (Consultant)

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WORK:

- Professor (Plant Protection Research Institute) – Alexandria- Egypt.
- Assistant professor: Pesticide chemistry, Plant Protection Department, College of Food and Agricultural Sciences, King Saud University.

EDUCATION:

- Ph.D. – Pesticide Chemistry
  Dept. of pesticide Chemistry, Alex University. 1992
- M.SC – Pesticide Chemistry, Dept. of pesticide Chemistry , Alex University. 1979
- B.Sc. – Pesticide Chemistry, Alex University. 1975

Visits and work outside Egypt

a. United States of America: 28-2-1993 to 28-2-1994.
b. Basra University: from 1981 to 1982.
c. King Saud University (Saudi Arabia): from 1985 to 1988.
d. King Saud University (Saudi Arabia): from 1994 until now.

RESEARCH INTERESTS:

Toxicity of pesticides against insects, rodents and snails
Biochemical effects of pesticides
Isolation of pesticidal compounds and extracts from plants

Teaching Career

- **Undergraduate level:**
  pesticide Toxicology and Chemistry

- General Pesticides
- Principles of Plant Protection
- Pesticide Formulation
- Environmental pollution

Graduate level

- Advanced Toxicology
- Pesticide Formulation (advanced)
- Chemistry of Pesticides (advanced)

Translated books:

The Pesticide Book
Authors: George W. Ware and David M.Whitacre 2004

Projects funded

5- Isolation of pesticides from Egyptian Plants Project NO 30, ministry of Agriculture
6- Pesticidal and antifeeding extracts from plants grown in Riyadh, Project NO 30Agric. Res. Cent., Ksu
7- Effects of Neemix 4.5 on *Spodoptera littoralis* and experimental mice, Project NO MS-4-38 KACST
8- Susceptibility of *Culex pipiens* to insecticides used in Riyadh City, Agric. Res. Cent., Ksu

PUBLICATIONS:

8. Ali S. Al-Sarar, Abdulwahab M. Al-Hafiz, Yasser Abo Bakr, Alaa E. Bayoumi and Hamdi I. Hussein (2011). Effects of Space Spray Application Methods on Fenitrothion Efficacy and Development of Resistance in *Culex pipiens***.** Journal of the American Mosquito Control Association, 27(2):000–000 (***ISI Indexed. Impact Factor: 0.906***)
9. Al-Sarar, A.S., A.W.M. Hafiz, A.E. Bayoumi, H.I. Hussein and Y.A. Bakr, 2011. Impact of fenitrothion thermal fogging on some biological and biochemical parameters in New Zealand rabbits as non-target organisms. *Int. J. Agric. Biol.*, 13: 435–438
10. Ali S. Al-Sarar1, Dafreen Al-Shahrani, Alaa E. Bayoumi, Yasser Abobakr And Hamdy I. Hussein (2011) Laboratory and Field Evaluation of Some Chemical and Biological Larvicides Against *Culex* spp. (DIPTERA: CULICIDAE**)** Immature Stages. International Journal of Agriculture & Biology, 13 (1): 115–119 (***ISI Indexed***)
11. Al-Sarar,A.S, Abo Bakr, Y, Al-Erima, G.S and Hussein, H. I.2009. Pesticides occupational exposure in Riyadh, Kingdom of Saudi Arabia: Knowledge, Attidutes and Practices. J. King Saud Univ., 21, Agric Sci. (1): 21-26.
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13. Al-Sarar, A. S., Hussein, H. I., Al-Shahrani, D., Bayoumi, A. E. and Abo Bakr, Y.2009. Efficacy of Three Pyrethroids Applied by Thermal Fogging and Ultra Low Volume against *Culex Pipiens* (Diptera: Culicidae). Submitted.
14. Al-Harby, H. A; Al-Rajhi, D and Hussein, HI. 2008. Pesticidal properties of three Saudi plants: J. Agric. Sci. Mansoura Univ. 33 (10): 7487-7492.

15. Abo Bakr, Y., E. H. Eshra and H. I. Hussein. 2007. *Calotropis procera* glycosides are more effective on *Eobania vermiculata* (Müller) than methomyl and other plant glycosides. J. Agric. Sci. Mansoura Univ., vol 32 (No 12): 10519-10527.
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18. Hussein, HI., Abo Bakr, Y. and E. H. Eshra. 2007. Molluscicidal and biochemical effects of two plant-glycosides against land snails. Journal of the Advances in Agricultural Researches, vol. 12, No 4: 667-677.
19. Hussein, HI, Eshra, E. H. and Y. Abo Bakr. 2007. Molluscicidal and biochemical effects of certain monoterpenoids against land snails. Journal of the Advances in Agricultural Researches, vol. 12, No 4 : 679-693.
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23. Hussein, HI; Al-Rajhi, D and Al-Assiry, M. 2005. Toxicity of four pyrethroid-based insecticides and kerosene to a laboratory population of *Culex pipiens*. Pakistan J Biological Sciences, 8 (5): 751-753.
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27. Al-Rajhi, D; Al-Ahmed, AM; Hussein, HI and Kheir, SM. 2003. Acaricidal effects of cardiac glycosides, azadirachtin and neem oil against the camel tick, *Hyalomma dromedarii* (Acari: Ixodidae). Pest Management Science, 59: 1250-1254.
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Letter of consent

I am pleased to confirm that I do agree to join the research teem of the proposed research project titled as **"Native mosquito larvicidal bacteria as new candidates in the battle against mosquito-borne diseases in Saudi Arabia"** for the period as stated in the proposal.

I thereby happy to be one of the consultants to help as needed any time at any stage of the research work during the proposal period.

Prof. Hamdy Ibrahim Hussein

Hamdy Hussein

Pesticide chemistry