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Biological Control of Insect Pests in Iraq: 2) an Overview of Microbial Control Research Development

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Abstract: Chemical insect pest control agents are extensively used in Iraq though they are regarded as ecologically unacceptable. Therefore, there is an increased pressure to replace them gradually with environmental friendly alternatives including entomopathogenic micro-organisms, such as bacteria, fungi, viruses and nematodes which are safe to humans and non-target organisms. In Iraq, such field of biological control of insect pests is progressing significantly. There are increasing volume of research programs conducted on several crops with formulations of *Bacillus thuringiensis*, *Beauveria bassiana*, *Metarhizium anisopliae, Baculovirus, Steinernema* and *Heterorhabditis* spp. for controlling several agricultural pests. This review outlines the current status and knowledge on the potential use of entomopathogenic micro-organisms in insect pests control in Iraq.

Key words: Biological Control · Entomopathogenic Micro-Organisms · Iraq

INTRODUCTION

The use of pathogenic micro-organisms for pest control is not a new concept. Attempts were being made as early as the late 19th century to use fungal diseases to control a number of economic pests. However, interest in all forms of biological and cultural control of insects declined with the dramatic impact of synthetic chemical pesticides in the 1940s and 1950s[1]. Current concerns over resistance to chemicals, non-target effects of pesticides and the cost of production of new compounds have renewed interest in alternative forms of pest control, among which disease causing micro-organisms hold out particular promise as part of Bio-intensive Integrated Pest Management programs. The most commonly used living organisms, which are pathogenic for the pest of interest, include entomopathogenic fungi (EF) (*Beauveria bassiana*), bacteria (EB) (*Bacillus thuringiensis*), viruses (EV) (nuclearplyhedrosis) and nematodes (EN) (*Steinernema* spp.) . The potential benefits to agriculture programs through the use of entomopathogenic micro-organisms (EMO) are considerable [2].

In Iraq, such field of biological control of insect pests was started during early 1960s, with humble attempts of utilizing commercial preparations of EB, *B. thuringiensis* and since then several problems limit the crop area where pathogenic micro-organisms are employed (will be illustrated in the last section). Hence, mentality of relaying upon pesticides as a magic solution is the most important element that limits the use of EMO, billions of dollars were spent on chemical pesticides in Iraq since 1960s, but not allocating enough financial support to survey, identify, formulation and field application of natural enemies (EMO) in well furnish facilities.

This review aims to show the progress accomplished in the field of BC technology research and development, mainly in this part the utilization of EMO (the first part was about parasites and predators[3]), by the Iraqi scientists and specialist since 1970s till the end of 2017. This will include some of the successful applications and finally the problems and challenges facing expansions and large implementations.

Present Status and Knowledge

Entomopathogenic Bacteria: *Bacillus thuringiensis* (*Bt*) is primarily a soil saprophyte bacteria and that association between *Bt* and insects is serendipitous [4]. In most cases ingestion of its toxin by insect is sufficient for death, though for some insects the presence of some spores is necessary for lethality [5]. Most commercial preparations are mixtures of spores and crystals. *Bt* is specific, killing only a limited range of insects (even Lepidopteron strains

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Table 1: List of entomopathogenic bacteria and insect pest species tested

Bacterial species	Insect species
Bacillus thuringiensis (Mostly *kurestaki*)	Peach worm, *Anarsia lineatella*
	Apple worm, *Laspeyresia pomonella*
	Almond moth, *Ephestia cautella*
	Dried fruit moth, *Ephestia calidella*
	Lesser grain borer, *Rhizopertha dominica*
	Lesser date moth, *Batrachedra amydraula*
	Potato tuber moth, *Phthorimaea operculella*
	Wax moth, *Galleria mellonella*
	Khapra beetle *Trogoderma granarium*
	Peach green aphid, *Myzus persicae*
	Cotton leafworm, *Spodoptera littoralis*
	Beet armyworm, *Spodoptera exigua*
	Black cutworm, *Agrotis ipsilon*
	Tomato borer, *Tuta absoluta*
	Termite, *Microcerotermes diversus*
	Carob moth, *Ectomyelios ceratoniae*
	Cabbage worm, *Pieris rapae*
	Cucurbit fly, *Dacus ciliatus*
	Cotton bollworm, *Heliothis armigera*
	Spiny bollworm, *Earias insulana*
	Cowpea podborer, *Etiella zinckenella*
	Corn stalk borer, *Sesamia cretica*
	House mosquito, *Culex pipiens, Aedes caspius* & *Theobaldia longiareolata*
	Southern house mosquito, *Culex quinquefasciatus*
Bacillus sphaericus	House mosquito, *Culex pipiens*
	Aedes caspius
B. laterosporus	House mosquito, *Culex pipiens*
	Aedes caspius
Bacillus carotarum	*Theobaldia longiareolata*
Bacillus cereus	*Theobaldia longiareolata*

do not kill all Lepidoptera with equal facility) while having no effect on birds, mammals and fish. This selectivity is environmentally advantageous but creates problems in an agricultural setting where a complex of pests has to be controlled. Other drawbacks include slow speed of action, per os activity and U.Vs. sensitivity [6].

In Iraq, researchers were start working with EB, specially *Bt* in mid 1960s, where [7] reported detailed study (for the first time) about the effect of *Bt* on spiny bollworm, *Earias insulana* in lab and field. In the 70s and 80s observations and field trials were also made on the effect of commercial formulations of *Bt* on peach worm, *Anarsia lineatella* [8], apple worm, *Laspeyresia pomonella* [9], *Ephestia cautella* [10], *Galleria mellonella* [11]. After that, utilization of *Bt* to control insect pests species was increased especially during period of 2010 till present time, where the number of published paper reached more than twenty articles dealt with more than ten pest species like tomato borer, *Tuta absoluta* [12], lesser date moth, *Batrachedra amydraula* [13]; corn stalk borer, *Sesamia cretica* [14], spiny bollworm, *Earias insulana* [15] and potato tuber moth, *Phthorimaea operculella* [16]. There were few articles dealt with *Bacillus* species to control mosquito larvae as a potential species transmitting malaria [17, 18]. Table 1 summarize the bacterial species utilized and the insect species tested.

The success cases in this field were as the following:

- The pioneered and comprehensive project concerned with isolation, identification, production, formulation and efficacy of *Bt* local isolates , was initiated at the Directorate of Agricultural and Biological Research, Iraqi Atomic Energy Commission during mid 1980s. The first *Bt* isolate was obtained from infected larvae of *Ephistia* spp. from rearing room and was identified as *Bt var. kaynea* by Pasture Institute, France, which was 1st record from Iraq [19]. Several experiments were conducted to determine the optimal conditions for growth and its efficacy (bioassays) using *Ephistia* larvae as a model and results were compared with that of commercial preparations. In 1991, patent was registered in Iraq about the best fermentation medium support the growth of different *Bt* isolates identified up to that time [20]. Field trials of lab scale produced formula indicated that it could protect date fruits at commercial warehouses from infestation by *Ephistia* spp. larvae for up to six months [21]. Moreover, lab formula efficacy was field tested upon lesser date moth, *Batrachedra amydraula* [22], Carob moth, *Ectomyelios ceratoniae* [23] and Cabbage worm, *Pieris rapae* [24]. The next step was transferred to the of pilot scale production of tow local *Bt* formulations as wetable powder (WP) using *kurstaki* variety which showed good efficacies and their potencies were 14500 and 12000 IU/mg for Alnaser II and Alnaser I (brand name of the product respectively [25]. In first half of the 90s and after several large scale field trials conducted in provinces of Sallah-Aldin, Waset, Babel, Dyalh and Baghdad to control corn stem borer, *Sesamia cretica* at early stage of plant development in cooperation with the State Board of Plant Protection at the Ministry of Agriculture, the product (Alnaser) was adapted by the Ministry of Agriculture to control this insect pest in whole fields of corn cultivation instead of synthetic chemical insecticide usually used at that time. The total amount supplied to the Ministry of Agriculture reached more than 50 tons up to 1996 after that every things stopped according to the UN inspection team.

- Large scale field application of the biological insecticide *Bt* in spray form, at a rate of 3 g/l (6-7 l/tree) to control lesser date moth, *Batrachedra amydraula*, was investigated [26, 27]. The results indicated that *Bt* was effective in reducing infestation of lesser date moth. However, the efficiency of the control was varied according to region being 79% in Basra and 35% in Al-Anbar. Good results were also obtained in Babil, Baghda, Diwanya and Wasit provinces. Other previous field trials showed that the efficiency of the biological insecticide *Bt* was about 66-75 during seasons of 2009 – 2011 [28, 29]. Trees treated with *Bt* resulted in various yield increase depending on location, cultivar and time of application. In addition, the efficacy of the egg parasitoid, *T. evanescens* and the bio-pesticide bacteria, *Bacillus thuringiensis kurstaki,* in reduction of lesser date moth, *B. amydraula* infestations were studied during seasons of 2011 and 2012 and the results were encouraging, thus an IPM project was developed for this purpose at the Ministry of Science and Technology, Directorate of Agricultural Research [30].

Entomopathogenic Fungi: Entomopathogenic fungi are important natural regulators of insect populations and have potential as mycoinsecticide agents against diverse insect pests in agriculture. These fungi infect their hosts by penetrating through the cuticle, gaining access to the hemolymph, producing toxins and grow by utilizing nutrients present in the haemocoel to avoid insect immune responses [31]. Entomopathogenic fungi may be applied in the form of conidia or mycelium which sporulates after application. The use of fungal entomopathogens as alternative to insecticide or combined application of insecticide with fungal entomopathogens could be very useful for insecticide resistant management [32].

Revision of the available published Iraqi literatures on utilizing entomopathogenic fungi indicated that the earliest article was of 1982 measured the efficacy of *Beauveria bassiana* on long horned date palm stem borer, *Jebusea hammerschmiditi* (*Pseudophilus testaceus*) [33]. The peak of activity was during 2000 till now, with total number of 95 published articles . Table 2 shows that the most fungal species studied was *Beauveria bassiana* followed by *Metarhizum anisopliae* and other entomopathogenic fungal species.

Different local isolates and few commercial preparations of *Beauveria bassiana* and *Metarhizum anisopliae* efficacies were studied on 40 different insect pests species, such as Mediterranean fruit fly, *Ceratitis capitata* [34], southern cowpea weevil, *Callosobruchus maculates* [35], sunn pest, *Eurygaster integriceps* [36], two spotted mite, *Tetranychus urticae* [37], peach green aphid, *Myzus persicae* [38], corn stem borer, *Sesamia cretica* [39], termite, *Microcerotermes diversus* [40] and dubas bug, *Ommatissus lybicus* [41].

The success cases in this field were as the following:

Two local isolates of *Beauveria bassiana* were isolated from long horned date palm stem borer.

Jebusea hammerschmidti, one (BJH) from Mahaweel area (Babel province, south of Baghdad) and the second (Bb) from date palm orchard soil in Basra province. The efficacy and pathogenesity of both isolates have been tested on different species of insects and mites, 1-10 days after spore spray. Both isolates showed 100% mortality after 5 days on cotton aphids, *Aphis gossypii* on cucumber, termites *Microcerotermes diversus*, scale insects, *Aonidella orientalis*, grape thrips *Retithrips syriacus*. The mortality reached 100% for green peach aphid, *Myzus persica* on potato, parlatoria scale insects, *Parlatoria blanchardi* on date palm and potato tuber

Table 2: List of entomopathogenic fugi and insect pest species tested

Fungal species	Insect species
Beauveria bassiana	Almond moth, *Ephestia cautella,*
	Mosquito, *Culex quinquefasciatus*
	Melon fly, *Dacus frontalis,*
	Mediterranean fruit fly, *Ceratitis capitata*
	Poplar leaf beetle, *Melasoma populi*
	Two spotted mite, *Tetranychus urticae ,*
	Cotton aphid, *Aphis gossypii,*
	Peach green aphid *, Myzus persica,*
	White scale insect, *Parlatoria blanchardi,*
	Oriental yellow scale, *Aonidiella orientalis,*
	Cotton white fly, *Bemisia tabaci,*
	Metalic borer beetles *Chalcophorella bagdadensis,*
	Large stem borer, *Capnodis miliaris,*
	Termite*, Microcerotermes diversus*
	Sunn pest, *Eurygaster spp.*
	Black vine thrips, *Retithrips syriacus,*
	Angomis grain moth, *Sitrotroga cerealella*
	German cockroach, *Blattela germanica*
	Mole cricket, *Gryllotalpa* sp.,
	Southern cowpea weevil, *Callosobruchus maculates*
	Lesser date moth, *Batrachedra amydraula,*
	Fig leaf worm*, Ocnerogyia Amanda,*
	Citrus mealy bug, *Planococcus citri,*
	Onion maggot, *Delia alliria,*
	Jasmini white fly *Aleuroclava jasmine,*
	Tuber moth, *Phthorimaea operculella,*
	Wax moth, *Galleria mellonella,*
	House fly, *Musca domestica,*
	Khapra beetle, *Trogoderma granarium,*
	long horned date palm stem borer, *Jebusea hammerschmiditi,*
	Corn stem borer, *Sesamia cretica,*
	Codling moth, *Cydia pomonella,*
	Cotton leaf worm, *Spodoptera littoralis*
	Dubas bug, *Ommatissus lybicus*
	Arabian Rhinoceros beetle, *Oryctes agamemnon arabicus*
	Fruit stalk borer, *Oryctes elegans*
	Lesser grain borer, *Rhizopertha dominica*
	Hyalopterus pruni
	Mealy Plum Aphid, *Aphis pomi*
	Broad bean black aphid, *Aphis fabe*
Metarhizium anisopliae	Saw toothed grain beetle, *Oryzaephilitis suriuamensis,*
	Mediterranean fruit fly, *Ceratitis capitata,*
	Mole cricket, *Gryllotalpa* sp.,
	Southern cowpea weevil, *Callosobruchus maculates*
	Tuber moth, *Phthorimaea operculella,*
	House fly, *Musca domestica,*
	Corn stem borer, *Sesamia cretica*
	Termite*, Microcerotermes diversus*
	Arabian Rhinoceros beetle, *Oryctes agamemnon arabicus*
	Fruit stalk borer, *Oryctes elegans*
	Lesser grain borer, *Rhizopertha dominica*

Table 2: Continued

Lecanicillium spp.	Soft scale insect, *Exaerotopus tritici,*
	Citrus mealy bug, *Planococcus citri,*
	Jasmini white fly, *Aleuroclava jasmine,*
	Termite*, Microcerotermes diversus,*
	White scale insect, *Parlatoria blanchardi,*
	Dubas bug, *Ommatissus lybicus*
	Cotton white fly, *Bamisia tabaci*
	Grain apid, *Schizphis graminum*
	Black legume aphid, *Aphis craccivora*
	Broad bean black aphid, *Aphis fabe*
	Grain English aphid, *Sitobion avenae*
	Oat aphid, *Rhopalosiphum padi*,
	Corn aphid, *R.maids*
Paecilomyces spp.	Poplar leaf beetle, *Melasoma populi,*
	Sunn pest, *Eurygaster integriceps,*
	Termite*, Microcerotermes diversus*
	Cotton white fly, *Bamisia tabaci*
	Broad bean black aphid, *Aphis fabe*
Tricoderma spp.	Cabbage aphid, *Brevicoryne brassicae*
	Grain apid, *Schizphis graminum*
	Lesser grain borer, *Rhizopertha dominica*
	Khapra beetle *Trogoderma granarium*
	Fig leaf worm*, Ocnerogyia amanda*
	Mole cricket, *Gryllotalpa* sp.
Isaria spp.	Mealy plum aphid, *Hyalopterus pruni*
	Apple aphid, *Aphis pomi*
	Citrus mealybug, *Planococcus citri*
Doratomyces stemonitis	House fly, *Musca domestica*
Emericella quadrilineata	House fly, *Musca domestica*
Zoophthora phytonomi	Alfalfa weevil, *Hypera postica*
Chaetomium elatum	Southern cowpea weevil, *Callosobruchus maculates*
Erynia phytonomi	Alfalfa weevil, *Hypera postica*
Alternaria alternate	Southern apple leaf worm, *Targama (streblote) siva*
Chaetomium sp.	Southern apple leaf worm, *Targama (streblote) siva*

moth, *Phthorimaea operculella* after 7 days of spore spray and on stone fruit borers, *Chalcophorella bagdadensis* and capnodis, *Capnodis miliaris* larvae and adults after 10 days. The mortality on sunn pests, *Eurygaster integriceps* was 100% after 7 days when sprayed by BJH and after 10 days for Bb however, it was 100 % after 7 days on mite, *Tetranychus spp.* for BJH and 10 days for Bb. Several solid and liquid production culture mediums have been tested and found that rice seed culture produced 3.2 x 10^8 spores /gm while potato sucrose broth and date fruit syrup (debis) cultures produced 5x10^7 and 3.9x10^7 spores/ml respectively [42].

The presence of natural endophytic *Beauveria bassiana* within date palm tissues using molecular technique and measure their field efficacies in controlling dubas bug, *Ommatissus lybicus* (Deberg) were investigated recently [43]. Two entomopathogenic *B. bassiana* isolates (MARD 108 and 100) were isolated from date palm, *Phoenix dactylifera* L. leaves; in addition, one isolate (MARD 92) originally isolated from soil was identified to have endophytic property. Concentration of 1 × 10^9 conidia/mL of each of three endophytic isolates was used in field experiments targeting dubas bug nymphs via injection tree trunks. The results indicated that the mortality rates reached 92%, 96% and 100% with infliction of the three endophytic isolates after15 d from the treatment. The successful establishment of the fungal isolates in the date palm tissue was determined using *B. bassiana* species-specific primer for the first time via using conventional polymerase chain reaction (PCR) amplification technique before and after injection and the positive gel band representation was the identification signs. The novel results depicted for the first time the presence of natural endophytic *B. bassiana* isolates within date palm tissues and their field efficacies in controlling dubas bug, *O.lybicus* (Deberg) infestation.

Entomopathogenic Viruses and Nematodes: First well-documented introduction of entomopathogenic virus into the environment which resulted in effective suppression of a pest occurred accidentally before the World War II. Along with a parasitoid imported to Canada to suppress spruce sawfly *Diprion hercyniae*, an nuclear polyhedrosis virus (NPV) specific for spruce sawfly was introduced and since then no control measures have been required against this hymenopteran species. In the past, the application worldwide of baculoviruses for the protection of agricultural annual crops, fruit orchards and forests has not matched their potential. The number of registered pesticides based on baculovirus, though slowly, increases steadily. At present, it exceeds fifty formulations, some of them being the same baculovirus preparations distributed under different trade names in different countries [44]

In Iraq, the published articles in this subject found to be limited (not more than 8 scientific papers). The earliest article was in 1999, which utilized local isolated granulosis virus to control tuber moth, *Phthorimea operculella* under field conditions [45] . Later, NPV was isolated and its efficacy on cotton leaf worm, *Spodoptera littoralis* was investigated [46 - 49]. Another study depicted the isolation of virus belong to family Entomopoxviridae infected long horn palm stem borer, *Jebusaea hammerschmidti* [50]. In addition, Oryctes -like virus was found infested larvae of palm stalk borer, *Orycts elegans* [51].

Nematodes that parasitize insects, known as entomopathogenic nematodes (EPNs), have been described from 23 nematode families [52]. Of all of the nematodes studied for biological control of insects, the Steinernematidae and Heterorhabditidae have received the most attention because they possess many of the attributes of effective biological control agents and have been utilized as classical, conservational and augmentative biological control agents. The vast majority of applied research has focused on their potential as inundatively applied augmentative biological control agents. Extensive research over the past three decades has demonstrated both their successes and failures for control of insect pests of crops, ornamental and lawn and turf. They can be considered good candidates for integrated pest management and sustainable agriculture due to a variety of attributes: some species can recycle and persist in the environment; they may have direct and/or indirect effects on populations of plant parasitic nematodes and plant pathogens; can play an indirect role in improving soil quality; and are compatible with a wide range of chemical and biological pesticides used in IPM programs [53].

Utilization of EPNs in controlling insect pests in Iraq back to late eighteens of the last century, when commercial preparations (as a gel) were brought from England and tested against onion fly, *Delia antiqua* and termite, *Microcerotermes diversus* infested citrus trees. The results were encouraged, hence the shutdown of the facility halted the project (Prof. Abdul-Sattar A. Ali (Al-Anbar Univ., Personal communication). The first documented isolation and identification of EPN *Steinernema* sp. in Iraq was from long horn palm stem borer and palm stalk borer [54].This nematode species was utilized through injection of date palm trunk with suspension of nutrient medium and nematode, the result indicated the presence of infected borer larvae with nematode after 3 months [50]. The result of field treatment of olive trees with the nematodes *S. carpocapsae* and *Heterorhabditis bacteriophora,* indicated that such treatment inflect high mortality in termite *Microcerotermes diversus* worker and could protect the trees for 3 months [55]. *Steinernema carpocapsae* was isolated for the first time from apricot stem borer, *Chalcophorella bagdadensis* [56]. Laboratory study was conducted to evaluate the efficiencies of entomopathogenic nematode isolated from 13 different sites around Baghdad, using concentrations of 0.0, 25, 50 infective nematode juvenile /5 larvae of stem corn borer *Sesamia cretica* [57]. Results obtained showed that all nematode concentrations used were efficient in infecting and causing high mortalities of larvae of stem corn.

Moreover, recently a new entomopathogenic nematode *Rhabdits blumi* (Nematoda: Rhabditida) was isolated from Arabian rhinoceros beetle (ARB), *Oryctes agamemnon arabicus* larvae [58] . Laboratory results demonstrated that direct spray of 1000 infective juveniles (IJs) per mL of *R. blumi* on (ARB), caused 71.67% and 15% mortality in the larvae adults respectively. Treating the food source of the larvae (pieces of fresh tissue of the frond bases) with the same dose and period resulted in 48.33% mortality in larvae and 10% in the adults. Field experiments results showed that injection of 50 mL per palm tree with a concentration of 1000 IJs/mL of *R. blumi* inflected about 42% mortality in ARB larvae infested the tree. Research, nowadays concentrated upon culturing, mass rearing and storing the nematode. Therefore, different culture mediums (lab formulated and commercial products from abroad) were tested and finally reached to choose a lab formulated medium which support efficiently both the reproductive and pathogenic capacities of the nematode. Furthermore, the lab formulated medium could facilitate storing the nematode for around five months without any deleterious effect on nematode pathogenicity (Mohammed Z. Khlaf, Ministry of Science and Technology, Baghdad, Personal communication).

Problems and Challenges Facing Implementing BC Programs in Large Areas in Iraq: In addition to the problems and challenges mentioned before[3], it could be adding the following:

- There are two main areas of safety issues that must be considered when implementing a biological control program. The first question that must be answered is the introduction of the biocontrol agent will have adverse effects on non-target organisms? The second concern is the strength and duration of the biocontrol agent on the environment.
- In order to increase the utility of microbial pathogens in IPM programs, systematic surveys are required in different agroecological zones to identify naturally occurring pathogens. Detailed studies are necessary on the properties, mode-of-action and pathogenicity of such organisms. Ecological studies on the dynamics of diseases in insect populations are necessary because the environmental factors play a significant role in disease outbreaks and ultimate control of the pests.
- Acceptance and implementation of the technology by farmers needs more efforts by specialist and extension personals in order to persuade the farmers about the benefit that they would obtain when using safe alternative. Farmers Field School could be one of the approaches.

Request for Information: It has taken me a lot of time to obtain data on the use of biological control in Iraq and I convinced that this survey is still not complete. I encourage Iraqi researcher to send me all what they have of information, so I can provide a more reliable overview in the future and build up the data base which will be established in the net.

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