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# FREQUENCY AND DISTRIBUTION OF *BACILLUS THURINGIENSIS* FROM ETHIOPIAN SOILS

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**ABSTRACT:** Search for *B. thuringiensis* with novel crystal proteins and effectiveness against insect pests from soils and other environments is underway worldwide. In the present study, *B. thuringiensis* were isolated from various agro-ecological soils of different zones in Ethiopia and larvicidal activity of isolates was determined using insect bioassay. Of the 503 soil samples examined, 32% yielded *B. thuringiensis. B. thuringiensis* was found to be distributed in all the studied agro-ecological soils. Tepid to cool semi-arid lakes and rift valley soils yielded *B. thuringiensis* more frequently, followed by cold to very cold sub-humid afroalpine and hot to warm perhumid lowlands. On the other hand, *B. thuringiensis* was least frequent in soils from hot to warm moist lowlands and hot to warm sub-moist midhighlands. It was also shown that vegetative cover influences the distribution of the bacteria in the soil. Two hundred and thirteen *B. thuringiensis* isolates were tested for larvicidal activity against *An. arabiensis* (Diptera) and *Plutella xylostella* (Lepidoptera) larvae. Of the tested isolates, 44 (21%) killed 50-100% of *An. arabiensis* larvae within 48 hours. Isolates that killed 100% larvae within 24 hours were all from tepid to cool moist midhighland soils whereas those which killed 100% larvae within 48 hours were from three other different agro-ecological soils. None of the 110 *B. thuringiensis* strains tested against *Plutella xylostella* larvae showed any larvicidal activity.

**Key words/phrases:** Agro-ecological soil, *Anopheles arabiensis, Bacillus thuringiensis*, Frequency of isolation, Larvicidal property, *Plutella xylostella*

## INTRODUCTION

*Bacillus thuringiensis* is a rod–shaped, gram positive bacterium that occurs in soil, water, dead insects, leaves of plants and grain dust (Martin and Travers, 1989; Cavado *et al.,* 2001). During sporulation, these bacteria produce a large crystal protein (δ-endotoxins) that is toxic against many insect pests and disease vectors (Hofte and Whiteley, 1989). *B. thuringiensis* was first discovered by Ishiwata in 1901 in Japan during a study of bacterial disease of silk worm "soto" (Ohba and Aizawa, 1986). In 1915, Berliner found a similar bacillus that killed larvae of flour moths in Germany and named it *Bacillus thuringiensis* after Thuringia, the region in Germany in

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which the disease occurred (Ohba and Aizawa, 1986). Since then, isolation of *B. thuringiensis* from soils and other habitats has attracted worldwide interest because of the bio-insecticidal property. Studies on the distribution of *B. thuringiensis* showed its association with geographic areas, soil types, plant communities, etc (Martin and Travers, 1989; Chilcott and Wigley, 1993). Consequently, collections of *B. thuringiensis* have been established from different ecological habitats around the world.

Ethiopia is known as a center of origin and diversity for many plant species (Abebe Demissie, 1998). Ethiopia's diverse climatic and agro-ecological conditions may also favor rich diversity of microbial flora. But, the hitherto studies on larvicidal activity of indigenous *B. thuringiensis* against disease vectors are limited (Aklilu Seyoum and Dawit Abate, 1997; Mogessie Ashenafi *et al*., 2000, 2001). Another study tested commercial preparations of *B. thuringiensis* var *kurstaki* against African bollworm, *Helicoverpa armigera* (Alemayehu Refera *et al*., 1993). The efficacy of standard *B. thuringiensis* subspecies *kurstaki* on Ethiopian crop pests was reviewed by Tsedeke Abate (1997). However, a thorough knowledge of the ecological distribution and diversity of *B. thuringiensis* would have a substantial contribution to evaluate their exact role in the environment and their effects on non-target organisms. It also helps to choose appropriate soil types and insect habitats to isolate novel *B. thuringiensis* strains.

The aim of the present study was, therefore, to evaluate the frequency of isolation and diversity of *B. thuringiensis* from different agro-ecological soils of Ethiopia and determine their biological activity against *An. arabiensis* (Diptera) and *Plutella xylostella* (Lepidoptera) larvae.

## MATERIALS AND METHODS

### Study Area

Ethiopia is located in East Africa between $3^{o}$ 24' and $14^{o}$ 53' North and $32^{o}$ 42' and $48^{o}$ 12' East. According to the Ministry of Agriculture (MOA, 2000), Ethiopia is divided into 18 major agro-ecological zones and 49 sub zones. The term "agro-ecological zone" is used to describe the broad temperature, moisture, and elevation conditions of an area. The major zones are sub-divided into sub-zones which are more homogenous in terms of climate, physiography, soil, vegetation, land use, farming system, and animals. In Ethiopia, the sub agro-ecological zones of the arid and semi-arid are less cultivated than the sub-moist, moist sub-humid and humid zones. This is because they receive less amount of rainfall and rain availability is less dependable. The agricultural lands are mainly sub-moist, moist, sub-

humid, and humid (MOA, 2000).

Out of the 18 major agro-ecological zones of Ethiopia, soil samples were collected from 16 accessible zones. At least one sub-zone from each agro-ecological zone was represented based on its accessibility. Characteristic features of the specific sub-zones including temperature and moisture regime is given in Table 1. Soil samples were taken in duplicate from different cultivated fields and natural vegetations (micro-habitat), which included forests and grazing lands.

**Isolation of microorganisms**

Surface soil was scraped off and about 200 grams of soil samples were taken from a depth of 4 to 10 cm with sterile spoon and transferred into sterile plastic bags. Ten grams of soil sample were added to 100 ml of sterile distilled water and agitated with magnetic stirrer for 10 minutes. One ml of the soil homogenate was serially diluted and heated in a shaker-water bath at $80^0$ C for 10 minutes to destroy vegetative cells and other microorganisms.

A volume of 0.1 ml of appropriate dilution was transferred onto Nutrient Agar (Difco) and spread plated using bent glass rod. The culture was incubated aerobically at $28^o$ C for 48-72 hours.

**Differential staining for crystal protein inclusions**

*B. thuringiensis*-like colonies were picked from 48-72 h old agar cultures. Smears were prepared and differentially stained following the method of Chilcott and Wigley (1988). Air dried smears were incubated at $100^o$ C in an incubator-oven for 10 minutes. The hot slides were then placed into naphthalene black solution (Naphthal black 12 B, in 35% V/V glacial acetic acid) for 2 minutes. The stained slide was washed with tap water and immersed in improved Gurr's R66 Giemsa for 1 minute. The slide smears were left in air to dry and then were observed with light microscope (1000X magnification). Vegetative cells and crystals stained black while spores stained pale to light blue. A direct observation of wet smear preparation was made under phase contrast microscopy. Refractile spores and their positions were seen with bright centers whereas crystals were faint. Phase contrast microscopy was confirmed using Differential Interference Contrast (Nowarski) Optics Microscope (1000x) fitted with camera and software.

Table 1 Agro-ecological zones studied versus some of their characteristic features

| Symbol | AEZ | Sampling Area | Mean annual temp. ($^{o}$C) | Mean annual rainfall (mm) | Soil type |
|---|---|---|---|---|---|
| A1 | Hot to warm arid lowland | Afar, Diredawa | >21 | 300-800 | Eutric, Regosols |
| H1 | Hot to warm humid lowland | Derashe | > 21 | 1200-1500 | Dystric Nitosols |
| H2 | Tepid to cool humid highland | Gore, Bedele, Metu, Jimma | 11-21 | 700-2200 | Dystric Nitosols |
| M1 | Hot to warm moist lowland | AsebeTeferi, Metahara | >21 | 600-1600 | Orthic Acrisols |
| M2 | Tepid to cool moist midhigh land | Ambo, Holeta, Fiche, etc | 11-21 | 1000-1800 | Vertisols, Nitosols |
| M3 | Cold to very cold moist sub afro alpine | Quarit, Amhara regions | 7.5-11 | 1000-1800 | Phaeozems, Leptisol |
| Ph1 | Hot to warm perhumid lowland | Bench Maji zone | 23.5-25.5 | 1100-1500 | Eutric Fluvisol |
| Ph2 | Tepid to cool perhumid midhighland | Keficho Shekicho zone | 11-21 | 1100-2200 | Dystric Nitosol |
| SA1 | Hot to warm sub moist midhighland | Humera | 21-28 | 300-800 | Vertisols |
| SA2 | Tepid to cool semi-arid lakes and rift alleys | Bulbula area | 11-21 | 600-800 | Vertic andosols |
| SH1 | Hot to warm sub-humid lowland | Bebeka, Arbaminch | >21 | 700-1000 | Vertisol, Fluvisol |
| SH2 | Tepid to cool sub-humid midhighland | Harari, Alemaya | 11-21 | 700-2200 | Dystric Nitosols |
| SH3 | Cold to very cold sub humid sub afroalpine | South west Chencha | 7.5-15 | 700-1500 | Humic Cambisols |
| SM1 | Hot to warm sub moist lowland | Metema | >21 | 200-1000 | Vertisols Cambisols |
| SM2 | Tepid to cool sub moist midhighland | Nazeret, Mojo, DebreZeit | 11-21 | 700-1200 | Vertic andosols |
| SM3 | Cold to very cold submoist subafroalpine to afroalpine | Feres Bet, Workamba | Not given | Not given | Haplic Phaeozems |

## Larvicidal activity

*B. thuringiensis* isolates from nutrient agar slants were transferred into fresh nutrient agar plates and incubated at 28$^{o}$ C aerobically until complete sporulation and lysis of all vegetative cells was achieved. Completion of sporulation and lysis was followed by observing a wet mount of the cultures

under bright field microscope. A loopful of completely sporulated colonies was transferred to 5 ml saline solutions (0.85% Nacl) in test tubes. The spore-crystal suspensions were adjusted to 0.5 McFarland standards ($1.5x10^8$ CFU/ ml). Bioassay was done against larvae of a malaria vector, *Anopheles arabiensis* (Diptera) and a plant pest, *Plutella xylostella* (Lepidoptera).

Larvae of *Anopheles arabiensis (*Diptera) were obtained from the insectaria of the Vector Control Unit of the Institute of Pathobiolgy, Addis Ababa University. Biological activity of spore–crystal complex against third instar larvae of *An. arabiensis* was tested by transferring 5 ml of spore-crystal suspension to each of a series of duplicate beakers with 10 larvae in 50 ml chlorine-free water. The larvae were then kept at room temperature for up to 48 hours. *B. thuringiensis* sub-species *israelensis* ATCC 35646 was transferred into duplicate beakers containing *An. arabiensis* larvae in chlorine-free water as positive control, and beakers containing larvae in chlorine-free water without spore-crystal suspension served as negative control. Death of larvae was checked by probing larvae with a wire loop. Number of dead larvae was recorded at 24 and 48 hours.

Larvicidal activity of our isolates was also assayed against *Plutella xylostella* (Lepidopteran) larvae at the Department of Entomology, SLU, Uppsala, Sweden. Third to fourth instar larvae of *Plutella xylostella* (Lepidoptera) were collected from cabbage cultivars *(Brassica olercea*) in the green house of the Department of Entomology, SLU, Uppsala, Sweden.

5 ml of spore crystal complex containing approximately $1.5x10^8$ CFU/ml was vortexed thoroughly and transferred into 30 ml sterile distilled water in a 50 ml plastic container. Leaflets from cabbage cultivars were used and a leaf–dip bioassay procedure previously indicated by Seal and Leibee (2003) was carried out. Each bioassay was conducted twice. Leaflets treated with *B. thuringiensis subsp. kurstaki* HD1 obtained from *Bacillus* Genetic Stock Center (Ohio State University, USA) were used as positive control and non-treated leaflets infested with larvae were used as negative control. Mortality counts were done at 48 and 72 hours.

## RESULTS

Diverse bacterial colonies were picked from 48 hour Nutrient agar culture plates. Big, dry and sticky colonies showed gram positive rods with refractile spores. Phase contrast microscopy revealed faint crystal protein inclusion, characteristic of *B. thuringiensis*. Average counts of colonies of spore formers from different agro-ecological soil samples ranged from

$8x10^2$ to $2.0x10^5$ CFU/g. Soil samples from tepid to cool sub-moist highlands (SM2) yielded highest colony counts followed by tepid to cool midhighland (M2) (Table 2).

Table 2. Frequency of isolation of *Bacillus thuringiensis* from soils of the studied AEZs

| AEZ | No. soil sample | Mean colony count (CFU/g) | Soil sample with Bt | % |
|---|---|---|---|---|
| A1 | 8 | $6.4x10^3$ | 3 | 38 |
| H1 | 6 | $8.3x10^2$ | 2 | 33 |
| H2 | 67 | $6.3x10^3$ | 19 | 28 |
| M1 | 13 | | 1 | 8 |
| M2 | 191 | $7.6x10^4$ | 52 | 27 |
| M3 | 20 | $8.1x10^2$ | 9 | 45 |
| Ph1 | 11 | $9.9x10^3$ | 6 | 55 |
| Ph2 | 35 | $3.7x10^4$ | 14 | 40 |
| SA1 | 21 | $1.2x10^3$ | 1 | 5 |
| SA2 | 9 | $9.5x10^3$ | 7 | 78 |
| SH1 | 21 | $8.6x10^3$ | 11 | 52 |
| SH2 | 33 | $6.5x10^3$ | 10 | 30 |
| SH3 | 13 | $6.0x10^3$ | 10 | 77 |
| SM1 | 26 | $2.7x10^3$ | 6 | 23 |
| SM2 | 9 | $2.1x10^5$ | 4 | 44 |
| SM3 | 20 | $1.2x10^3$ | 6 | 30 |
| Total | 503 | | 161 | 32 |

All the 503 soil samples collected from 16 agro-ecological zones yielded various colony types belonging to *Bacillus* species. Of these, 220 (43.7%) soil samples yielded bacterial isolates that were identified as *B. cereus group* based on colony morphology. Out of the 220 samples, 75.6% were found to have crystal inclusion forming bacilli. *B. thuringiensis* was isolated from 32% of the total soil samples. Over 50% of the soil samples from tepid to cool semi-arid lakes and rift valleys (SA2), cold to very cold sub-humid sub-afroalpine (SH3), hot to warm perhumid lowland (PH1) and hot to warm sub-humid lowland (SH1) yielded *B thuringiensis.* The frequency of isolation from soil samples for most agro-ecological zones ranged between 5% and 45% (Table 2). The highest frequency (78%) was observed in tepid to cool semi-arid lakes and rift valleys (SA2) samples. Diverse vegetation types such as roots and tubers, sugar cane, oil seeds, etc., collectively yielded the highest proportion of Bt (67%) (Table 3). Soils from fruit fields and cultivated crops also had high frequency of isolation of *B. thuringiensis*. From among cereal covers, soils from barley and wheat yielded 47% (10/21) and 36% (8/22) *B. thuringiensis,* respectively. Soils from natural vegetation covers produced 39% (35/93).

Table 3 Vegetation cover of soils vs. *Bacillus thuringiensis* isolates from AEZs of Ethiopia

| Vegetation cover | No of soil samples | No of soil samples with Bt | % |
|---|---|---|---|
| Teff | 59 | 16 | 27.1 |
| Wheat | 22 | 8 | 36.4 |
| Barely | 21 | 10 | 47.6 |
| Maize | 43 | 5 | 11.6 |
| Sorghum | 35 | 10 | 28.6 |
| Legumes | 54 | 14 | 25.9 |
| Mixed crops | 8 | 2 | 25.0 |
| Vegetables | 49 | 12 | 24.5 |
| Fruits | 6 | 3 | 50.0 |
| Chat | 7 | 2 | 28.6 |
| Coffee | 21 | 6 | 28.6 |
| Sesame | 10 | 3 | 50.0 |
| Cotton | 12 | 2 | 16.7 |
| Enset (*Ensete ventricosum*) | 15 | 6 | 40.0 |
| Nug (*Guizotia sp.*) | 15 | 4 | 26.7 |
| Others, tubers,oil seeds etc. | 33 | 22 | 66.7 |
| Natural vegetation | 93 | 36 | 38.7 |
| Total | 503 | 161 | 32.0 |

A total of 213 *B. thuringiensis*-like colony, selected on the basis of phenotypic characteristics were screened for their biological activity on *An. arabiensis.* A total of 12 isolates killed 100% of the larvae within 24 hours and other 12 isolates killed the larvae within 48 hours (Table 4). Other 13 isolates showed 70-95% killing of the larvae (Table 4). The remaining isolates killed from less than 40 % to 70 % larvae within 48 hours. These larvicidal *B. thuringiensis* strains were isolated from almost all the agro-ecological zones studied, but strains that killed 100% larvae within 24 hours were all from tepid to cool agro-ecological zones (M2). Strains that killed 100% of larvae within 48 hours were from different agro-ecological zones. The positive control with *B. thuringiensis* subsp. *israelensis* also resulted in killing 100% of the larvae in 24 hours while no larva was killed in the negative control.

None of the strains were found active against the tested *Plutella xylostella* insect larvae. The control strain *B. thuringiensis* subspecies *kurstaki* HD1 killed 100% of the larvae in 48 hours.

Table 4 *B. thuringiensis* isolates that killed *An. arabiensis* larvae within 24-48 hrs

| AEZ | Strain | % larvae killed (24 h) | % larvae killed (48 h) |
|---|---|---|---|
| M2 | FGB2 | 100 | - |
| - | FGJB | 40 | 40 |
| - | GDMC | 100 | - |
| - | DKF | 100 | - |
| - | BYW2 | 100 | - |
| - | GMAS | 55 | 65 |
| - | FWAW | 90 | 90 |
| - | GIAT2 | 90 | 90 |
| - | GAS | 65 | 80 |
| - | GDBm | 95 | 95 |
| - | GDFW2 | 100 | - |
| - | GIP | 100 | - |
| - | ISGP | 90 | - |
| - | FGJW2 | 85 | 85 |
| - | KWB2 | 95 | 100 |
| - | DEP2 | 100 | - |
| - | DDN | 100 | - |
| - | FGW | 100 | - |
| - | FGT | 100 | - |
| - | DGS | 100 | - |
| - | FWO | 100 | - |
| SH1 | BBGB | 45 | 50 |
| - | BBGB2 | 70 | 70 |
| - | DTAM | 75 | 100 |
| - | DTAG | 80 | 100 |
| - | AKC | 90 | 100 |
| SM3 | GDFT | 85 | 95 |
| - | GWMN | 50 | 55 |
| - | GDFb | 70 | 90 |
| - | GWMN2 | 90 | 90 |
| M3 | QDT2 | 90 | 95 |
| - | QGT2 | 95 | 95 |
| H2 | JGSn | 50 | 95 |
| - | IBKT | 55 | 95 |
| - | IYM | 90 | 100 |
| - | IGDN | 65 | 100 |
| - | JDGI2 | 70 | 100 |
| - | IBKT2 | 75 | 95 |
| - | JGHS2 | 95 | 100 |
| - | JGP | 55 | 100 |
| - | JGTN | 60 | 100 |
| SH2 | SDI | 90 | 100 |
| - | DMAB2 | 60 | 100 |
| PH2 | SMGB | 75 | 75 |
| PH1 | BSWC | 70 | 70 |

## DISCUSSION

In this study, sampling sites were selected on the basis of vegetation cover, which varied in terms of cultivated crops and natural vegetation. All soil samples, however, had at least a sample containing *B. thuringiensis* and this is indicative of the distribution of *B. thuringiensis* all over the agro-ecological zones although the isolation was not equally frequent in all soils. The frequency of *B. thuringiensis* was highest in tepid to cool semi-arid

lakes and rift valley soils (SA2) followed by cold to very cold sub-humid afroalpine (SH3) and hot to warm perhumid low lands (Ph1). SH3 is characterized by limited plant species due to its cold temperature whereas the other two zones are characterized by moderate to rich fertility, which may be associated with several factors such as soil nutrients and physical and biological components important in spore germination and recycling of *B. thuringiensis*. *B. thuringiensis* was least frequent in soil samples collected from hot to warm sub-moist midhighlands (SA1) and hot to warm moist lowlands (M1). Several workers (Hossain *et al.*, 1997; Nicholson, 2002) have suggested that variation in abundance and distribution of *B. thuringiensis* in the soils of different sources might be due to several ecological factors that affect the viability of *B. thuringiensis* spores and recycling of *B. thuringiensis* either by growth at the expense of nutrient present in the environment, or, to some extent, by association with diversity of insect types which may be distributed in those agro-ecological zones.

The 32% *B. thuringiensis* isolation in the present study was much lower than the 70% isolation rate from New Zealand (Chilcott and Weigley, 1993), 82 % from Colombia (Uribe *et al*., 2003), 90% from Mexico (Bravo *et al.*, 1998) and 100% from Bangladesh (Hossain *et al*., 1997). Nevertheless, our isolation rate was markedly higher than 2.7% from Japan (Ohba and Aizwa, 1986), and 0.5 % from United States (DeLucca *et al*., 1981).

Another source of variation in the frequency of isolation of *B. thuringiensis* may be related to media used for isolation as pointed out by different workers (Martin and Travers, 1989; Chilcott and Wiegley, 1993; Hossain *et al.,* 1997). Chilcott and Wiegley (1993) however commented that a more important measure of success for isolation of *B. thuringiensis* is to rely on good sources of *B. thuringiensis* such as soil type, insect habitats, and insects than the isolation medium being used. Thus the relatively lower isolation rate in our case may be due to the low abundance of Bt in the studied area.

The present data showed that soil samples from different crop fields vary in harboring *B. thuringiensis.* For instance, barely fields harbored *B. thuringiensis* more frequently than wheat fields from among cereal crop covers. Soil samples from the natural vegetation yielded *B. thuringiensis* in a comparable rate to soils of cultivated fields. Soil samples collected from vegetable fields had lowest *B. thuringiensis* yield. It is generally known that the number and types of microorganisms in soil vary depending on the type

of plant cover. Naturally, insect pest distribution also differs with vegetation cover (Janzen, 1973; Bianchi *et al*., 2006), and if *B. thuringiensis* distribution has any correlation to crop pest distributions, it is apparent that its distribution and abundance will differ with vegetation types of the soil. Although the physical and chemical properties of the present soil samples were not analyzed, the study of Hossain *et al.* (1997) clearly demonstrated that the properties of soil affect the abundance and distribution of *B. thuringiensis*. In our study, we observed some differences in frequency of isolation of *B. thuringiensis* from one agro-ecology to another. However, it cannot be said which specific factors influenced the distribution in the agro-ecological soils. Hence, further examination on the association of the physico-chemical properties of agro-ecological soils of the country with abundance and distribution of *B. thuringiensis* may be required.

The result also showed that 44 (21%) of the isolated spore forming bacilli with crystal inclusion morphology killed 50-100% of *An. arabiensis* (dipteran) larvae. There were differences in efficacy of the *B. thuringiensis* isolates against the tested larvae. Although the larvicidal strains were found in all agro-ecological zones, those that killed 100% of the larvae within 24 hours were localized in a specific agro-ecological zone (M2), and those that killed 100% of the tested larvae in 48 hours were isolated from three different zones. Martin and Travers (1989) indicated that *B. thuringiensis* sub-species were specifically distributed in different soils. These researchers observed that toxicity to certain types of insects appeared to be clustered in some samples. For instance *B. thuringiensis kurstaki* types were found to be most common from soils in Asia, while *B. thuringiensis* sub species *israelensis* or similar biochemical types were common in African and Central American soils.

The biological activity of *B. thuringiensis* against *An. arabiensis* in the present study is comparable to the activity of *B. thuringiensis* strains against mosquito species studied previously. A preliminary screening for larvicidal property in Ethiopia showed that 39 (30%) of *Bacillus* isolates exhibited activity against *Aedes africanus* (Mogessie Ashenafi *et al*., 2000, 2001). Martin and Travers (1989) found that 22.5% of their isolates were active against mosquito larvae, whereas Chilcott and Wigley (1993) recorded 48% of their isolates to be active against mosquito larvae. Ohba and Aizawa (1986) tested *B. thuringiensis* isolates from soils of Japan against larvae of *Aedes aegypti* and found that 10.6 % of the isolates resulted in 70-100% mortality. However, a comparison would be more appropriate if insects belonged to the same species since sensitivity differs from one species to

another within a genus (Jaquet *et al*., 1987). Some workers have compared the susceptibility of mosquito species and indicated that, in general, species of *Aedes* and *Culex* were more sensitive than species of *Anopheles* to *B. thuringiensis* subsp. *israelensis* (Porter *et al.*, 1993). A study from Ethiopia compared the efficacy of *B. thuringiensis* subsp. *israelensis* against *An. arabiensis* and *Culex quinquefaciatus* and reported that *An. arabiensis* was more susceptible than *Cx. quinquefaciatus* (Aklilu Seyoum and Dawit Abate, 1997). In the present study, we used *B. thuringiensis subsp. israelensis* as a positive control. Similar to that of the standard *B. thuringiensis* subsp.*israelensis*, twelve of our isolates were equally efficient to kill 100% of the tested larvae within 24 hours. Many studies have demonstrated that *B.thuringiensis subsp.israelensis* and *B. sphaeri*cus are very effective against Diptera. Thus, this preliminary screening bioassay may indicate that these strains are related to the subsp. *israelensis* and other Dipteran active toxin producing *B. thuringiensis.*

None of the crystal inclusion-containing *B. thuringiensis* tested against *Plutella xylostella* (lepidopteran) larvae in the study were toxic. Although none of our isolates were toxic against lepidopteran larvae, we need to test them against a variety of other lepidopteran species of economic importance before we rule them out for control purposes. Many workers have recorded a large number of *B. thuringiensis* isolates to be non-toxic against lepidoptera insects (Ohba and Aizawa, 1986; Meadows *et al.,* 1992). Many other investigators have also reported that a large number of their *B. thuringiensis* isolates showed no activity against Lepidoptera, Diptera, or Coleoptera, and they suggested that these isolates were novel strains which may be active against other insects not yet tested (Ohba and Aizawa*.,* 1987; Lamber *et al*., 1992; Chilcott and Wigley, 1993; Bravo *et al*., 1998).

Insecticidal resistance is now a major problem facing malaria vector control programs in most African countries as the three important vector species, *An. gambiae*, *An. arabiensis,* and *An. funestus*, show resistance to one or more of the insecticide classes used in vector control programs (Coetzee, 2003). Thus it is important to provide alternatives for coping with problems of insect resistance. *An. arabiensis* is the major malaria vector in Ethiopia followed by *An. phroensis, An. funestus* and *An. nili* (Coetzee, 2003; Meshesha Balkew *et al.,* 2003). It has been reported that these mosquito species are developing some degree of resistance to traditional insecticidal substances such as DDT (Coetzee, 2003; Meshesha Balkew *et al.,* 2003). Therefore, our finding of indigenous *B. thuringiensis* strains that are highly active against *An. arabiensis* may have a positive impact on future malaria

vector control programs.

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