ADDIS ABABA UNIVERSITY SCHOOL OF GRADUATE STUDIES DEPARTMENT OF BIOLOGY

Study on Ovicidal, Larval Settlement, Feeding and Oviposition Deterrence Effects of Seed Kernel Powder Extracts of Birbira, (*Milletia ferruginea*) on Diamondback moth, (*Plutella. xylostella* L.) (Lepidoptera: Plutellidae)

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Acronyms

AA	Acetic acid
AC	Acetone
AMAS	Australian Ministry of Agricultural Service
AVRDC	Asian Vegetable Research and Development Center
CH	Chloroform
EIAR	Ethiopian Agricultural and Research Institute
FAO	Food and Agricultural Organization
HARC	Holetta Agricultural Research Center
HE	Hexane
IFAS	Institute of Food and Agricultural Science
IGRs	Insect Growth Regulators
NCFAP	National Center for Food and Agricultural Policy
SARDI	South Australian Research and Development Institute
TO	Toluene
US	United States
WA	Water

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Abstract: Solvent extractions having different polarities with different doses prepared from birbira tree seeds, *Milletia ferruginea* (Hochst) Baker. This study was carried out to assay the ovicidal, larval settlement, feeding and oviposition deterrence effects on Diamondback moth (*Plutella xylostella* L.). Leaf dipping method was used to run both assays by using the extracts from birbira seed kernel powder in water (w/v): 0, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, and 1.4 mg/ml. In the assays significantly higher larval mortality was recorded on leaves treated with polar than the non polar and Chloroform extracts. Days to the 1st larval mortality was observed within a day after the exposure of the 3rd instar larvae on treated leaves with different solvent extracts of birbira except the Water. Days to the last mortality were observed on the 14th days on all extracts. Days to the highest larval mortality were significantly higher for Water, Acetic acid and Acetone, extracts at 0.8, 0.6 and 0.4 mg/ml; respectively for the other extracts it was significantly higher at the higher rates of application. In the control most larvae pupated on 8th day. Acetone prevented pupation at the higher rates, Water, Acetic acid, Chloroform and Hexane allowed fewer larvae to pupate, and in contrast more number of larvae was pupated in Toluene extract at all the rates. Significantly small numbers of adults were emerged at the higher rates of Water, Acetic acid and Acetone than the others. Adults took longer time to emerge from pupae on Chloroform, Toluene and Hexane extracts at the higher rates. Eggs unhatchability was higher at 0.4 mg/ml and increased at the rate of 1.4 mg/ml for polar extracts. However, significant eggs unhatchability was recorded only at the higher rates for the non polar extracts. Oviposition deterrence was significantly lower at the lower rates of Water, Acetic acid and Acetone full deterrence was recorded at the higher rates, in contrast Toluene and Hexane showed least deterrence effect even at the higher rates of application. The number of eggs oviposited in the inner surface of the Petri dishes were higher at all the rates except for Water, Acetone and Chloroform extracts. Numbers of eggs deposited in the inner walls of the cages were higher at 0.6 and 1.4 mg/ml for Water and at 0.4 and 1.4 mg/ml for Hexane. Small numbers of larvae were settled on non polar solvent extracts. Mortality caused by extracts with Water, and Acetone was very high, and have much smaller LC_{50} values. In contrast the LC_{50} was higher for the other extracts and caused significant larvae kill only at the higher rates. Thus, results of this study indicate that *M. ferrugginea* has a good possibility for controlling *P. xylostella* (L.).

1. Introduction

Vegetables particularly Cabbage, *Brassica oleracea* L. *var Capitata*, Kale, *B. oleracea* L. *var acepbaba*, Cauliflower, *B. oleracea* L. *var botrytis*, Ethiopian mustard, *B. carinata*, Chinese Cabbage, *B. campestris* L. *var pekinensis* and broccoli, *B. oleracea* L. *var italica* are the major crucifer vegetables grown for home consumption and for cash in many parts of the world, notably in highlands (Nyambo and Löhr, 2006). Cabbage and cauliflower are important vegetable cash crops to low income farmers throughout Asia, Africa, Latin America and the Caribbean. In such countries like Ethiopia where the people experience malnutrition due to heavy dependence on cereals such as tef (*Eragrostis tef*), maize (*Zea mays*), wheat and other cereals, vegetables are important for food security in times of drought, famine and food shortage. They provide a source of income for the farmers/producers; create employment opportunity and contribute to the national economy as export commodities (Fekadu Mariame and Dandena Gelmesa, 2006). Subsistence farmers gain revenue upon sale of the vegetables in the local market and thus it is an important food and cash crops in the rural and urban areas in back yard culture.

They are also important sources of vitamins and minerals for human health throughout Tropical and Sub-Tropical areas of the World (Bert, 2006). Cabbage is an annual vegetable with nutritional, medicinal and economic benefits. It contains vitamins, minerals, proteins, carbohydrates, fats and fibers. Eighty five grams of boiled vegetables (cabbage) contains 1.3 gm of protein, 1.5 gm of carbohydrates, and 0.1gm of total fats and 1.4 gm of fiber (Reuben et al, 2006). It is used daily in dishes as a relish and curative benefits such as in the control of bronchitis, cataracts, diabetes, crohn's disease, diverticular disease, heart attack and hypertension. Glucosinolates are 'S' containing natural products primerly from the Brassicaceae (Mustard family). Some epidemiological data support the possibility that glucosinolates breakdown products derived from brassica vegetables (Cabbage, broccoli and relatives) may protect against human cancer, especially in the

gastrointestinal tract and lung (Johnson, 2003). It is also an excellent source of calcium, folic acid, vitamin C, and potassium (Roes and Chapman, 2005).

Productions of crucifers including cabbage is often constrained by damage caused by a range of insect pests such as Diamondback moth (*Plutella xylostella* L.), aphid (*Brevicoryne brassicae* L.), flea beetle (*Phyllotreta sp.*), cabbage leaf miner (*Chromatomyia horticola*) (Goureu), cabbage looper (*Trichoplusia ni* (Hubner) and cut worms (Agrotis species) (Abate and Ayalew, 1992). Among these, DBM is the most important destructive insect pest of cruciferous crops throughout the world (AVRDC, 1997; Facknath, 1997; Ebrahimi, et al., 2009).

Diamondback moth belongs to the order Lepidoptera and family plutellidae. This moth is small, grayish, brown and has pale white narrow wings with inner margins yellow. When the wings are folded on its body while at rest a diamond shaped median dorsal patch is seen and hence the common name "diamondback moth" (Nayar, 1992). The habitat of this pest is ubiquitous, and is found wherever cabbage and related vegetables are grown. The pest is oligophagus with the larvae feeding specifically on membranes of the cruciferae family that contain mustard glycosides (Talekar and Shelton, 1993). The larvae feed on the parenchyma leaving the cuticle intact but as the plant grows, the cuticle tears resulting in damaged leaf surface hence rendering the vegetables unsuitable for human consumption or sale (Capinera, 2009). It is considered as the most important limiting factor of successful production of cruciferous vegetables, particularly in the tropics. In the warmer humid tropics, the pest breeds throughout the year and can have more than ten generations annually and entire crop can be lost if control measures are not taken (Facknath, 1997).

Crop damage and loss due to feeding by DBM larvae is estimated to total billions of dollars each year in the US alone, with comparable losses in many other developed countries. The loss is even more staggering in the developing countries mainly in Asia and Africa (Jacobson, 2000). In Ethiopia, reported yield loss on cabbage from the pest ranges between 36.1% and 91.2% corresponding to 12 to 48.7 tons/ha respectively

(Gashawbeza Ayalew, 2006). Total crop failure is common in season of heavy infestation in central rift valley areas (Ayalew and Oogal, 2006).

Control of this pest largely depends on the use of synthetic insecticides. Most of the chemical control in crucifers' production is directed towards Diamondback moth control. Globally over a billion of US dollars are spent on pesticides each year to control this pest (Talekar and Shelton, 1993). Since in the warmer and humid climatic conditions Diamondback moth population multiplies fast, it has been forcing farmers to rely on the use of synthetic insecticides, which is the quickest way to check pest populations (AVRDC, 1997). Though, chemical pesticides have been effective, the pest has progressively developed resistance to many of the insecticides such as Diazinon, Fenvelarate, Endosulfan Cypermethrin, Permethrin and Fiproni (Prijono, 2004).

DBM is the first crop pest in the world to have developed resistance to the synthetic pesticide, DDT. To date, it has developed resistance to all modern insecticides in many countries that have been used intensively including some toxins of the bacterium *Bacillus thuringensis* (Bt) (Henry and Baker, 2008). In South Africa, wide spread and indiscriminate use of pesticides on DBM population has shown resistance to synthetic pyrethroides, organophosphates and carbamates. However, farmers in Africa largely depend on the use of insecticides for the control of this pest. These chemicals are frequently applied and thus uneconomical to achieve effective control (Lohr and Gathu, 2002).

Continuous, heavy usage of synthetic insecticides over the years has created serious problems, the most obvious being direct toxicity to non target organisms such as parasitoids, predators, pollinators and man. The problem of insecticide resistance as well as environmental and consumer health hazards associated with insecticide residue in plant materials have recently changed the attention of scientists towards looking for alternative methods for the control of DBM. This has encouraged research and led to testing of plant extracts as botanical pesticides.

Most botanicals are safe to prepare and apply, cheaper and selectively more effective than synthetic pesticides. The safety nature of most botanicals has been attributed to the faster decomposition in sunlight and high temperature, leaving no residual effect in water, the crop or after cooking (FAO, 2000). After world war 2nd synthetic pesticides, such as DDT, increased in popularity and the use of botanical pesticides rapidly declined. However, in recent years the problems associated with the use of synthetic pesticides has resulted in a renewed interest in the use of plant extracts to control *P. xylostella*. Investigation into alternative control mechanisms for the control of DBM has led to the testing of plant extracts. Of 1800 plant species reported by Graingu et al (1984) cited in Charleston, (2004) to posses' pest control properties, more than 80 species have been reported to be effective against DBM. The Meliacea family and others including Asteraceae, Fabaceae and Euphorbiaceae contain most of the insecticidal plant species reported. The botanical insecticide widely used against *P. xylostella,* pyrethrum and rotenone were isolated from plant species belonging to the Asteraceae and Fabaceae, respectively (Reuben et al., 2006 and Jacobson, 2000).

Extracts from birbira, *Militia ferruginea* (Hochst) baker have been made from seed kernel powder and have been found to give good control of *P. xylostella* (Bayeh and Brook, unpublished 2009). Assaying the ovicidal, larval settlement, feeding and oviposition deterrence effect of Birbira may help to control Diamondback moth, because both aqueous and organic extracts from birbira have been found to contain substances with insecticidal potency. Crude extracts from seeds of birbira were found to be effective on storage and field insects (Bekele et al., 2002). Extracts from the seeds of birbira are easily prepared from locally available trees and is short lived in the environment. It was found that both aqueous and organic extracts were found to be effective. Implying that the active insecticides are several or single that can dissolve in solvents with different property (Bayeh and Brook, unpublished 2009). This has prompted the investigation on crude extracts that could be obtained using solvents of varying polarity.

Therefore, the insecticidal potency of extracts should have been tested on insects with different feeding habit. Diamondback moth is exposed feeder on crucifers and is easily

reachable by contact. Therefore being a chewing insect it is at high risk of dying due to exposure to botanical extracts directly applied on leaves while feeding. Therefore the insecticidal potency of Birbira was investigated by studying the ovicidal, settlement, feeding and oviposition deterrence effects of Birbira seed kernel extracts obtained using solvents of different polarity.

2. Objectives of the study

2.1. General objective

- The main objective of this study is to evaluate the efficacy of extracts from *Millettia ferruginea* seed kernel powder using different solvents for the possible IPM option of Diamondback moth (*Plutella xylostella* L.) management.

2.2. Specific objectives

- To determine feeding deterrence effect of different extracts from *Millettia ferruginea* seed kernel obtained with solvents of different polarity.
- To determine oviposition deterrence effect of different extracts from *Millettia ferruginea* seed kernel obtained with solvents of different polarity.
- To determine ovicidal effect of different extracts from *Millettia ferruginea* seed kernel obtained with solvents of different polarity.
- To determine the larval settling response effect of different extracts from *Millettia ferruginea* seed kernel obtained with solvents of different polarity.
- To identify the most efficient solvent that may extract the potent insecticides present in *Millettia ferruginea* seed kernel.

3. Literature review

3.1 Biology of Diamondback moth, *Plutella xylostella* (L.)

Diamondback moth is a lepidopterous species that reproduces sexually and undergoes complete metamorphosis. The life cycle of DBM is dependt on the prevailing temperature and typically it completes its life cycle in about 25-30 days (Capinera, 2009).

Egg stage: Diamondback moth eggs are small (<1 mm), white, roundish or oval, and somewhat irregular shaped flattened approximately 0.4 mm long, 0.2 mm wide and yellow to pale green. Eggs are laid either singly or in small clusters on the upper and lower side of leaves, often along the mid-rib or on both sides of the principal veins of the leaves. Cordero and Kuhar (2009) reported that eggs are deposited singly or in small groups of two to eight eggs on leaves or other parts of the plant, the junction of leaf veins on the upper surface of the leaves tends to be an attractive ovipostion site. Eggs hatch in approximately 5 to 6 days under normal field conditions and its incubation period is 3 to 8 days depending on the temperature on the top of the leaf.

Larval stage: Diamondback moth larvae (caterpillars) are pale green, slightly tapered at each end and grow through four stages with a dark head in the 1st instars and green with greenish brown head in the final instars and fully grown up larvae measures 10-12 mm (Henry and Baker, 2008). Compared to other caterpillars in cruciferous crops, mature DBM larvae are small. The larvae are colorless in the first instar, but thereafter are green. the body form tapers at both ends, bears relatively few hairs, which are short in length, and a pair of protrudes prolegs from the posterior end, forming a distinctive ‘v’ and the prologs are five in pairs (Capinera, 2009).

When disturbed, DBM larvae often wriggle violently moving backward and spin down from the plant on a strand of silk. They climb back again on the leaf on this thread once the danger has passed (Cardero and Kuhar, 2009). Soon after emergence, larvae start feeding on foliage. The larvae undergo three moultings to pass through four instars (growth stages), the first instar larvae initially wander over the leaf surface and feed as miners where as older larvae feed from the lower leaf surface by scraping the epidermis of the crucifer plant and usually consume all tissue except the wax layer on the upper

surface, thus creating a 'window' in the leaf (Henry and Baker, 2008). Larvae development occurs within 10 to 14 days depending on temperature.

The freshly hatched larva and full grown larvae measure on average 1.30 x 0.18 mm and 8.62 mm in length respectively. Average and range of development time of the four instars larvae is about 4.5 (3-7), 4 (2-7), 4 (2-8), and 5 (2-10) days, respectively (Bhalla and Dubey, 2006). Total larval period averaged 11.3 days (range 9 to 13 days) in the first and 10.3 days (range 9 to 12 days) in the second generation (Cordero and Kuhar, 2009). Once mature, the larvae spin a loose, open lacework white cocoon that is attached to the leaves or stems of the plant or spun under the plant for pupation.

Pupal stage: The newly formed pupa is approximately 7 to 9 mm long, remains active and yellowish green, but in a day or two it becomes brown in color until the time of adult emergence (Capinera, 2009, Bhalla and Dubey, 2006). Pupation occurs in a loose silk cocoon usually formed on the lower or outer leaves. Thereafter, it shortens its body longitudinally but remains active. The pupal stage requires from 5 to 15 days to complete development (Cordero and Kuhar, 2009).

Adult stage: Adult DBM is small, slender, long, and grayish-brown moths with pronounced pair of antenna and folded wings flaring outward and upward at their posterior ends. When the moths are viewed from the side, the tips of the wings can be seen to turn upwards slightly. They are distinguished from others by having three pale, triangular markings along the inner margins of the wings. When the moth is settled at rest, these join together to form a row of three diamonds shaped pale spots down the middle of the back; which is the base for the common name of this pest 'Diamondback moth' (Mike et al., 2004).

Adult DBM emerge one week later after pupation or in 7 to 15 days during the evening and rarely in the morning hours. Mating of adult DBM occurs on the day of emergence. Cordero and Kuhar (2009) reported that adults started copulating almost immediately after emerging from pupa because female produce a strong sex pheromone, which attract the male. Oviposition takes place in the evening and during the night. Oviposition period lasts 3-10 days depending on environmental condition and peak oviposition occurs on the

1st day of emergence (Belay Beyene, 2007 unpublished). Usually 50-150 tiny white eggs are laid and the majority of eggs are laid before midnights with peak oviposition (Talekar and Shelton, 1993). Eggs are laid preferentially in the cavities of leaves than on the smooth surface. A female moth lay minute, yellowish white Eggs singly as many as 57 Eggs on the undersurface of a leaf along the veins (Mike et al., 2004). Temperatures, trichomes, and waxes on leaf surface all influence oviposition (Talekar and Shelton, 1993).

Male DBM is dark brown with three white diamond shaped patterns aligned on its back. The female is tan colored and its diamond patterns are less distinct than those of the male (Endersby et al., 1996). Adults of Diamondback moth can live four, seven or eight weeks, but the usual life span is around two weeks. Adult males and females live about 12 to 16 days respectively, and 3 to 6 days without food, and for 11 to 16 days provided with foods (Salinas, 1986).

The life processes of this insect are highly influenced by environmental conditions. In the temperate during winter, the pest develops much more slowly but as the temperature increases in spring and summer, the moth goes through its life cycle more quickly. In one summer, the moth will complete 6-72 lifecycles. According to Henry and Baker (2008) there is considerable overlap in generations, with a stage being present at any one time. Endersby et al., (1996) reviewed that generation overlap throughout the warmer months of the year may be up to 10 generations in one year, and numbers can multiply very rapidly. In tropical climates, with host plants available throughout the year, more than 20 generations of diamondback moth may be produced each year.

Distribution

The origin of DBM was believed to be in the Mediterranean region, however it was first reported in North America in Illinois in 1854 (Gianessi, 2009). Diamondback moth is an insect of European origin, is now widespread throughout the world, causing damage to all economically important cruciferous vegetables (AVRDC, 1997). DBM is an oligophagous insect and will feed on plants that contain mustard glucosides (Ooi, 1983 and Capinera, 2009). Plants with mustard glucosides are members of family Cruciferae,

which are essentially temperate climate crops. However, the crucifers, in particular the genus *Brassica,* have been spread from their original home to other regions. It is very likely that DBM spread along with the spread of the crucifers.

DBM is now recorded everywhere that cabbage is grown and is highly dispersive. This pest thrives well in tropical, subtropical and temperate climates. Under warmer and humid climatic conditions, it develops rapidly throughout the year with new generations emerging every two to four weeks. Consequently pest densities increase significantly in a very short period of time (Lohr and Gathu, 2002). Now DBM is the most important cruciferous pests worldwide and is often difficult to control.

Host plants

The host plant for DBM includes both wild and cultivated plants in the family cruciferea. The association of DBM to crucifer species is due to the presence of one or more glycosinolates, singrin, sinalbin and glycocheirolin which act as specific feeding stimulant (Talekar and Shelton, 1993). Cabbage and Cauliflower are the most preferred hosts by DBM. Head cabbage, *Brassica oleracea var. capitata* L., was the most suitable host with the shortest developmental period and the highest reproductive potential (FAO, 2000). When compared to other crops, DBM preferred to oviposit eggs on cabbage, which was followed by cauliflower (*B. oleracea* L. subsp. *Botrytis*), broccoli (*B. oleracea* L. subsp. *italica*), and kohlrabi (*B. oleracea* L. subsp. *gnongylodes*). This host preference may be due in part to different chemical cues possibly involved in the acceptance or rejection of potential hosts (Reddy,et al., 2004).

Host preference studies at the Colorado State University (2003) have shown that DBM prefers cabbage over both cauliflower and broccoli, and cauliflower over broccoli. In this study they observed that most female DBM orientated toward cabbage and cauliflower in a wind tunnel bioassay. The results of this study showed that DBM completed larval and pupal development in the shortest time on cauliflower, cabbage, and radish, and survival was greatest on these crops as compared to turnip and mustard. Studies on host preference of DBM by AVRDC (1997) in Shanhua (Taiwan) showed that larvae

consumed less foliage and required more time to complete development on kohlrabi and kale, thereby indicating that these cruciferous crops were the least accepted hosts.

Several cruciferous weeds are also important hosts especially early in the season before cultivated crops are available (Capinera, 2009). Cruciferous weeds, such as wild mustard are important food sources early in the spring before crops are planted (Gianessi et al., 2002 and Gianessi, 2009). DBM maintains itself on these weeds only early in the absence of more favored cultivated hosts.

Behavior

Diamondback moth larvae are generally found on the underside of the leaves, between the veins or in the developing flower buds. They can burrow into the leaves when they are small, making small white tunnels. Later, they feed on the underside of the leaves when they become large. Larvae of DBM can be distinguished from other young pest species by their habit of actively wriggling or dropping from the leaf on a silken thread when disturbed. They climb back again on the leaf on this thread once the danger has passed. The newly formed pupa remains active and yellowish green but in a day or two it becomes brown in color until the time of adult emergence (Bhalla and Dubey, 2006).

Adult moths prefer to rest under the leaves and in protective plant structures. They move rapidly when disturbed, and flight is usually in the form of quick fluttering from plant to plant. During the day if plants are disturbed the moth would be seen flying from their resting places (Talekar and Shelton, 1993). Although this activity occurs during the day, moths' activity is greatest at dusk and down. The moth is weak flier, usually flying within two meters of the ground and not flying long distance. However, they are readily carried by the wind especially when host plant materials are dried up and the moths have to disperse to survive (Capinera, 2009).

Damage and economic importance of DBM

DBM is the most destructive insect pest of numerous cruciferous crops worldwide and severely limited their production, especially in resource-poor regions (Talekar and Shelton, 1993). According to AVRDC (1997) report the pest's damage is especially

serious in the tropics where host plants and ideal temperature for insect development and multiplication exist throughout the year. DBM can cause damage particularly throughout the year except for a brief period during the rainy season. Although most pests attack leaves and other fleshy, edible above ground plant parts, DBM is by far the most destructive and economically important throughout the world (Talekar, 1996).

Damage is caused by larval feeding on leaves, or by the presence of larvae, which contaminate and lower the quality of the product. The larvae can cause damage to all developmental stages of the plant. They generally feed on foliage before flowering. As flowering progresses, an increasing proportion of the larvae move to the floral buds, flowers and pods. Young pods may be fully eaten by the larvae; maturing pods are usually surface grazed (Henry and baker, 2008). The usual symptoms of DBM damage are small transparent, papery, "windows" on the surface of leaves; these windows do not go all the way through the leaf (Rowell, 2004). The immature instars larvae damage cruciferous plants by feeding and mining, mature instars larvae feed on leaf surface or on lower leaf epidermis, causing characteristic transport patches. The leaf mines of the newly hatched larvae appear as characteristic, pale white traces. The feeding of the larger larvae on the underside of leaves causes holes, often with the upper surface intact, which produces a window effect.

Although they are small in size relatives to other lepidopteran pests such as Cabbage looper, (*Trichoplusia ni*,) and imported cabbage worm, (*Pieris rapae*) densities of DBMlarvae can reach levels that result in total damage of leaves. The larvae feed voraciously on the exposed cabbage leaves causing direct damage to the marketable product (Gianessi, 2009). *P. xylostella* is a common pest in broccoli production, the presence of larvae in florets can result in the total rejection of the product (Capinera, 2001). Its infestation levels can reach as high as 100% of the fields. In 1997, there was an outbreak of DBM in California broccoli that resulted in a crop loss estimated to be more than $6 million (Gianessi et al., 2009).

The report of Jacobson (2000) showed that crop damage and loss due to feeding by insect larvae and adult is estimated to total billions of dollars each year in the US alone, with

comparable losses in many other developed countries. The loss is even more staggering in the developing countries mainly in Asia and Africa. In India, losses of cabbage and cauliflower due to Diamondback moth can reach 90% if not sprayed and 35% even if sprayed (Sandur, 2004). In Ethiopia, Gashawbeza Ayalew (2006) reported that yield loss on cabbage from the pest range between 36.1 and 91.2% corresponding to 12 to 48.7 ton/ha respectively. Total crop failure is common in seasons of heavy infestation in central rift valley areas (Ayalew and Ogoal, 2006).

Most of the chemical control in crucifer production is directed towards DBM control. However, in some part of the world, economic production of crucifer crops has become increasingly difficult due to insecticide failure to control this pest (Metcalf, 1980). This pest now occurs wherever crucifers are grown and control of this pest is estimated to cost the world economy about US 1 billion yearly (Talekar and Shelton, 1993).

3.2. Management of DBM

The management of DBM ranges from cultural control to the recent and novel techniques such as the use of pheromones, insect growth regulators (IGRs), resistant varieties, botanicals and biological control with the use of natural enemies and pathogens.

Cultural techniques

Cultural practices are manipulations of the cropping environment to increase pest mortality or reduce rates of pest increase and damage. Good cultural control methods often become stable farming practices that serve multiple purposes (Hein, 2003). The basic principles of cultural control methods are to manage the pest immediate environment so that there is disruption of the life cycle of the pest. Some of the common techniques that have been tried with some success are: Improving plant vigor with fertilization and water, tillage practices that disrupt the insect's life cycle and destruction of crop residue, changing of planting dates to minimize insect impact, and crop rotations that include non-susceptible crops.

Rotation: It is very effective practice for controlling the diamondback moth. a significant reduction in the numbers of caterpillars can be achieved by having a break of 6 weeks or

more where no crucifers are grown at all (HDRA, 2000). Continuous planting of crucifers allows continuous generations of DBM that leads to frequent use of insecticides and the development of pesticide resistance, crop rotation may become a necessity (Hama, 1990)).

Intercropping: It is an ancient and traditional agronomic practice. It involves planting two or more crops simultaneously or sequentially in various raw arrangements within a field. Specific plants may be chosen as intercrops because they restrict or inhibit the pest insects' ability to locate and colonize the main crop (Taleker and Shelton, 1993). The intercrops were grown in alternate rows with cabbage, and their influence on *Plutella* population was estimated. Apart from lowering infestation, the intercrops can also provide additional revenue to the grower. It was observed that the intercropped plots had significantly lower numbers of *Plutella* larvae and pupae, and a higher yield of good quality cabbage heads, as compared to the control (pure stand) cabbage plots (Facknath,1996).

The reduction in ovipostion and subsequent development of the pest was essentially due to emission of volatile compounds by intercrops. Several field trials have been conducted using different intercrops such as tomato, garlic; etc. Tomato exhibited the greatest deleterious effect on pest populations. Cabbage when intercropped with tomato has been reported to inhibit or reduce DBM oviposition (Talekar et al. 1992). Similarly, garlic in inter-rows of cabbage also has been reported to decrease *Plutella* numbers. Intercropping cabbage with garlic has been reported in America (Anderews et al., 1992).

Irrigation: Frequent irrigation and rain reduce the mating related flight activity of moths and wash off caterpillars and pupae from plant leaves. Overhead irrigation has been reported to reduce DBM injury on crucifers. This reduction is believed to be caused by drawning or physically dislodging of the insects from the plant surface (Talekar and Shelton, 1993).

Trap crops: *P. xylostella* has been reported as showing a distinct preference for mustard for oviposition. This attraction and oviposition stimulant property of mustard has been attributed to the effect of volatile compounds such as isothiocyanates released by the

mustard plants (Gianessi, 2009). Mustard attracts *Plutella* and other crucifer pests, thereby drawing them away from the main crop (Facknath, 1997). Trap crops such as mustard and yellow trap have been investigated for control of Diamondback moth. Mustard was tested for its role as a 'trap crop'. In India, Srinivasan and Krishna (1992) cited in Reddy et al., (2004) reported that when trap crops such as mustard was altered with every 15 rows of cabbage; DBM colonized the mustard and spared cabbage the main crop. Alternate rows of mustard and cabbage had lower numbers of *Plutella* larvae and pupae on the cabbages as compared to those on the pure stand cabbage.

Yellow rocket, *Barbarea vulgaris var. arcuata* proved to be a good candidate as a trap crop for diamondback moth because it is highly attractive for egg laying but larvae do not survive on it. However, larvae from other insects, such as imported cabbageworm, are able to develop successfully on yellow rocket (Gianessi, 2009). Prijon (2004) reported that the more viable approach would be to use a trap crop that the insect pests prefer to oviposit on but on which their offspring could not survive. For such an approach *Barbarea vulgaris var. arcuata* has a great potential as a dead end trap crop for improving management of DBM.

The activity of natural enemies may be enhanced by the presence of alternative host (trap crops), because adult parasitoids may respond to the presence of food sources, other than their pray, such as flower. Rape (*Brassica campestris var. oleifera*) and 'Sawi jabung' (*Brassica juncea*) can be used as trap crops for *Plutella xylostella* and can enhance the functions of the parasitoid, *D. semicalusum* because flowers of these plants provide nectar for the parasitoid adults (Reddy et al., 2004).

Adjustments of planting time: Cabbage planted in November to February (the rainy season) will be less attacked by *Plutella xylostella* than in the other seasons (Prijon, 2004). Studies on the bionomics of DBM in northwestern Himalaya in 2006 showed that it was found seriously damaging cabbage seed crops during dry cold September to October. However, the pest was observed feeding only in small numbers on cabbage and cauliflower grown as off-season vegetables during the wet summer months (June through September) and on cauliflower seed crops from December to May.

Mechanical/Physical Control Method

Yellow sticky traps: These are often made from local materials such as yellow colored pieces of plastic or cardboard. They can also be made from empty yellow plastic bottles in which engine oil is sold, or even from pieces of bamboo or wood that have been painted yellow. The trap should be painted or smeared with something sticky. Often glue or grease is used. The yellow color attracts *P. xylostella*. The trap is especially useful for monitoring the adult population (what direction they are coming from, and how abundant they are). To a lesser degree, it can be used as a control measure, to catch adults. However, not only pest insects are attracted to the yellow sticky traps but also some beneficial natural enemies. Thus, it would be advisable to place just one trap as a trial and monitor in detail which insects are caught. If large numbers of natural enemies stick to the glue it might be better to remove the traps (FAO, 2000).

Use of Resistant Varieties

Crucifer crops differ somewhat in their susceptibility to attack by DBM. Mustard, turnip and kohlrabi are among the more resistance crucifers, but resistance is not as pronounced as it is for imported cabbageworm and cabbage looper (Young et al., 2008, Capinera, 2009). Rauf et al., 2004 reported that white cabbage of rotan f-1 variety and red cabbage of Morner Rocco variety are somewhat tolerant to *P. xylostella*. The report of Bhalla and Dubey (2006) showed that during dry cold September to October the pest was observed feeding only on cabbage and cauliflower, no other popular cruciferous crops such as turnip, radish, knolkhol, kale, and mustards were found infested by the pest in the region. Several researchers have evaluated commercial cabbage cultivars for their susceptibility to cabbage looper damage. Some cultivars have a low level of resistance to the cabbage looper, but this resistance is inadequate as a control by itself (Gianessi, 2009).

Talekar and Shelton (1993) from their review showed that, several studies have surveyed existing germplasm for plant resistance to lepidoptera including, Diamondback moth. Moreover, they summarized that varieties also differ in susceptibility to damage by DBM and a major component of their resistance. Capinera (2009) also reviewed that the presence of leaf wax glossy varieties, lacking the normal waxy bloom and therefore green

rather than grayish green, are somewhat resistant to larvae of DBM. Therefore larvae apparently spend more time searching, and less time feeding, on glossy varieties. Cabbage lines derived with the glossy leaf trait have shown a high degree of resistance resulting from leaf-surface waxes. However, in cabbage, glossy leaf is undesirable because of lack of consumer acceptance.

There is a successful trial in producing resistant crucifers through transgenic carrying (*Bacillus thuriengensis*) Bt genes, to feeding by larvae of Diamondback moth. In 1995, researchers at Cornell University, Washington successfully used the bacterium *Agrobacterium tumefaciens* to transform five broccoli cultivars to produce an insecticidal protein [cry1a(c)] from the bacterium *Bacillus thuringiensis* (Bt). Bt-susceptible DBM larvae suffered 100% mortality when feeding on the transgenic broccoli (Gianessi, et al., 2002).

Use of Pheromones and Pheromone Traps

The sex pheromone emitted by female DBM has been identified as a three component mixture containing (z) 11- hexadecenal, (z) 11- hexadecenylacetate, and (z) 11-hexadecenyl alcohol and is commercially available (Chisholm et al., 1997: cited in Belay Beyene, 2007). High concentration of pheromone has been used for mating disruption of DBM in cabbage fields in Japan, but the strategy is not cost effective. In US, successful control of DBM achieved with mating disruption pheromone supplemented with some insecticide application (Talekar and Shelton, 1993). However, Schroeder et al (2000) reported that mating disruption of DBM was not effective even under controlled condition. Populations are usually monitored by making counts of larvae, or by the level of damage. Pheromone traps can be used to monitor adult populations, and may predict larval populations 11 to 21 days later. However because of variation among locations, each crop field requires independent monitoring (Capinera, 2009).

Insect Growth Regulators (IGRs)

The use of insect growth regulators (IGRs) is the newest of all approaches to operational and commercial insect control. Chitin synthesis inhibitors (i.e. of cuticle formation) and

substances that interfere with the action of insect hormones (i.e. JHs, ecdysteroids) are the major groups of insect growth regulators. IGRs adversely affect insects by regulating or inhibiting specific biochemical pathways or processes essential for insect growth and development. Some insects exposed to such compounds may die due to abnormal regulation of hormone-mediated cell or organ development. Others may die either from a prolonged exposure at the developmental stage to other mortality factors (susceptibility to natural enemies, environmental conditions etc) or from an abnormal termination of a developmental stage itself (Phillips and Throne, 2010).

Insect growth regulators may come from a blend of synthetic chemicals or from other natural sources, such as plants. For instance, the Neem tree (*Azadirachta indica* [A. Juss]) has been revered for possessing remarkable pest control properties due to the presence of azadirachtin (AZA). Structurally, AZA resembles the molting hormone, ecdysone. Research has shown that the presence of AZA in the insect body causes hormonal imbalances, which result in unsuccessful molts (i.e., death), or heavily deformed immature stages. In addition, AZA in some adult insects causes sterility and reduces egg-laying in females. AZA has also been shown to slowly paralyze the mouthparts of many species of insects, which causes them to die slowly of starvation (anti-feeding effect). The active ingredient (Ecozine) in azadirachtin of Neem tree at the rate of 8 to 10oz per acre is recommended to be used in the control of key pests such as diamondback moth (AMVAC, 2001).

A number of novel insecticides have recently been registered for the control of DBM. In California, Grafton-Cardwell et al., (2005) reported that Indoxacarb, tebufenozide, methoxyfenozide, and emamectin benzoate are novel insecticides that act as IGRs in the control of DBM. A major advantage of these new products is that they act on insect biological processes such as molting. Many also have greater selectivity to target specific species, so they are less likely to harm natural enemies and non-toxic to honeybees when compared with other insecticides.

Tebufenozide (dibenzoylhydrazine) and Methoxyfenozide (dibenzoylhydrazine) are stomach poisons; they mimic a molting hormone and block the completion of the normal

molting process. The insect stops feeding within a few hours and undergoes a premature lethal molt within 3 to 7 days, becoming trapped within the shedding head capsule. Tebufenozide must be ingested to take effect and is thus slow-acting, with a residual activity of 14 to 21 days. However, application timing is critical, because it is more active on early larval stages. Indoxacarb (oxadiazine insecticide) blocks the sodium channels in insect nerve cells, causing DBM larvae to stop feeding within 4 hours, become paralyzed and die within 2 to 5 days. It is more effective as a stomach poison than as a contact poison. Emamectin benzoate is a second-generation Avermectin analog with exceptional activity against DBM, acting by decreasing the excitability of neurons. Shortly after contact or feeding exposure, larvae stop feeding, become irreversibly paralyzed and die in 3 to 4 days. It has broad spectrum than methoxyfenozide, tebufenozide or indoxacarb, it kills a wide variety of lepidopterans (Grafton-Cardwell et al., 2005).

Interestingly, IGRs are effective against DBM cause the rapid death of the insect through failure of a key regulatory process to operate or function. IGRs generally control insects either through regulation of metamorphosis or interference with reproduction (Phillips and Throne, 2010). Compounds developed to disrupt metamorphosis ensure that no reproductive adults are formed. Those that specifically interfere with reproduction may include the development of adults with certain morphogenetic abnormalities that reduce their reproductive potential. Adults may be sterile or possess abnormally developed genitalia, which hinders the mating process or the capacity to produce fertile offspring (Tunaz and Uygun, 2004).

Use of Insecticides

There are several information available in different parts of the world on the chemical control of DBM. Also every year more information appears on new products and methods. This is probably due to the species becoming more resistant to the commonly used insecticides. In fact, as early as 1953, DBM was the first lepidoptera reported to be resistant to DDT and later to the microbial insecticide *Bacillus thuringiensis* (Indris and Norhayati Abdul Mukti, 1997). Throughout the world DBM has developed resistance to

many insecticides. The resistances of DBM to 36 insecticides in 14 countries have been reported by Georghiou (1981) (cited in Hill and Foster, 2000).

Factors which help contribute to the rapid development of resistance by DBM include their high fecundity, their rapid turnover of overlapping generations and the frequent use of insecticides in some Brassica cropping systems (Henery and Baker, 2008). Resistance to synthetic pyrethroids and organophosphates is widespread in DBM populations. In many regions of the world, classes of insecticides, including organophosphate, carbamates, organochlorines, pyrethroids and some botanicals no longer were able to control DBM (Indris and Norhayati Abdul Mukti, 1997). In all Australian states resistance to synthetic pyrethroid insecticides has been detected in populations of Diamondback moth. Since 1993, brassica growers in Victoria have had difficulty in controlling the caterpillars of Diamondback moth and have experienced insecticide control failures. However, insecticide control remains the major method of DBM control for commercial growing large-scale farmers despite the pesticide resistance development in DBM.

Use of insecticide is the most common method to suppress the pest outbreaks. In different countries of the world farmers use different kinds of chemicals such as Diazinon, Malthoion 85% and Bioprotec CAF and Bioprotec 3P (Bt ssp. Kurstaki) to control this pest. Although natural enemies provide a substantial amount of control of cabbage caterpillars, they often do not keep populations below economic threshold level and supplemental control through the use of a foliar-applied insecticide is necessary (Gianessi, 2009). Knowledge of toxicity, hazard, efficacy and application methods is critical to effective and safe use of insecticides (Triplehrn and Johnson, 2005). Overuse of some types of insecticides, however, can kill DBM parasitoids and make control more difficult. This overuse causes health problems for farmers applying the pesticides, may contaminate soil and water, and may result in excessive residues in vegetables. In addition, natural enemies of DBM and other pests are killed by this over-reliance on pesticides (Rowell, 2004).

In tropical areas where pest pressure is high, it is not uncommon to apply insecticides every other day. Such intense use of insecticides poses hazards to farmers, consumers and the environment. Moazami (1996) has reviewed that control of pest insects with chemical pesticides generated several problems including insecticide resistance, outbreaks of secondary pests normally held in check by natural enemies, safety risks for humans and domestic animals, contamination of ground water, decrease in biodiversity, and other environmental concerns.

Biological Control of DBM

Biological control is a natural phenomenon-the regulation of plant and animal population by its natural enemies. Talekar 1996 defined it as the action of parasites, predators, and pathogens in maintaining another organism's density at a lower average than would occur in their absence. In nature every organism has natural enemies and DBM has more than its fair share of predators, parasites and pathogens.

Lim (1992) lists 4 major egg parasitoids, 38 larval parasitoids, and 13 Pupal parasitoids that attack this pest worldwide. In addition 25 species of predators are reported to attack DBM. At least 2 viruses, 2 bacteria and 7 major species of fungi are pathogenic to this pest. Among all the species of parasites, five species are especially important. These include *Diadegma semiclausum* (Hellen), *Cotesia plutella* (Kurdjnmov), *Oomyzussokolowskii* (Kurdjnmov), *Diadromus collaris* (Gravenhorst) and *Microplitis plutellae* (Muesebeck). Establishment of one or more of these species in an area can give satisfactory control of DBM on a sustainable basis without the use of pesticides (AVRDC, 1997). Henry and baker (2008) reported that a number of natural enemies attack DBM. The most important of these are small wasp parasitoids-*Diadegma semiclausum, Apanteles ippeus* and *Diadromus collaris*. They lay eggs in the larvae or pupae of DBM and the developed adult wasp later emerges, killing the DBM host in the process. Biological control using the parasitoid *D. semiclausum* presently constitutes the backbone of IPM against *P. xylostella* and of cabbage IPM as a whole. This parasitoid can adequately control *P. xylostella* population in highland areas when insecticides are not intensively used. The activity of this parasitoid, however, is often badly interfered with by intensive use of insecticides (Bahalla and Dubey, 2006).

A few species of the polyphagous egg parasitoids belonging to the genera *Trichogramma* and *Trichogrammatoidae,* contribute little to natural control and require frequent mass releases. However, it gave satisfactory control in Thailand when imported by AVRDC from south East Asia country to test for the control of DBM (FAO, 2000). Larval parasitoids are the most predominant and effective. The success of using parasitoids, especially larval parasitoids like *Diadegma, Cotesia* and *Microplitis* species for controlling DBM have been reported in several countries. These larval parasitoids of DBM are commonly found in the field. *Diadegma semiclausum* (=eucerophaga) Hellen is the major parasitoid of DBM in Europe and Asia, while *Diadegma insulare* (Cresson) predominates in the Americas.

Many of the effective larval parasitoids are host specific and belong to two major genera, *Diadegma* and *Cotesia* (=Apanteles); a few *Diadromus* species, most of which are pupal parasitoids also exert significant control. The majority of these species come from Europe where DBM is believed to have originated. Because of the presence of natural enemies in Europe this pest is not considered a serious pest of crucifer in that continent. South east Asia, Pacific Islands, Central America, the Caribbean and most of the sub-Saharan Africa are constantly plagued by DBM because these area lack effective larval parasitoids (Talekar and Shelton, 1993).

There are several species of parasitoids, predators and pathogens that attack all stages of DBM populations in the field. However, most attention has been paid to natural enemies (parasitoids) of insect pests; undoubtedly both predators and pathogens also play important roles in pest population dynamics. Predators such as night birds and bats feed on moths, brown lacewing larvae (*Micromus tasmaniae*), several predacious bugs (e.g. *Nabis tasmanicus*), and a range of spiders, larvae of hover flies feed on DBM eggs, larvae and pupae and further contribute to their mortality (Henry and Baker, 2008).

Outbreaks of the disease *Zoophthera radicans* can also cause greater than 90% reduction in DBM population density. Diseased larvae become yellowish, sluggish and somewhat swollen before death. After death, their flattened, whitish, brittle bodies, are covered with fungal growth and remain attached on the plant surface. A combination of rainfall, high

humidity and warm temperatures are required for a *Z. radicans* disease outbreak (Henry and Baker, 2008). To date, the bacterium, *Bacillus thuringiensis* subspecies Kurstaki, has been the most widely used DBM pathogen. Although it is environment friendly pesticide, its effectiveness always varies with weather, field location and frequency of application per season. These are the main factors that contribute to the slow acceptance of *B. thuringiensis* by the cabbage growers worldwide. Because of this many studies have been done to improve its efficacy and persistence in the field.

Researchers have studied the use of entomopathogenic Nematodes for controlling the Diamondback moth. In field trials, Nematodes provided 41% control. The study concluded that repeated applications of Nematodes will probably be ineffective in attaining control. They require high humidity, moderate temperatures, and protection from ultraviolet light to be most effective (Gianessi, 2009).

Botanical control

Some plants containing chemicals that are toxic to insects when extracted from plants, these chemicals are called botanicals (FAO, 2000). Generally botanicals breakdown more rapidly than most conventional pesticides, they are considered relatively environmentally safe and less likely to kill natural enemies than insecticides with longer residual activity therefore, they are good alternatives to chemical pesticides. Botanical insecticides contained in fruits, leaves, root and especially seeds. It works as a stomach poison, contact poison, repellent and antifeedant. It is said to be effective against leafhoppers and caterpillars (FAO, 2000, Ignacimuthu, 2004). In many studies, a large number of local plant groups have been investigated for their pesticidal properties against a range of agricultural pests. According to Facknath (1997) plant species exhibited strong pesticidal potential against DBM, but their effect were varied.

Table below showed Plant species exhibiting pesticidal potency against *Plutella xylostella* L.

Plant species Common name	Scientific name	Biological activity	References
Ayapana	*Ayapana triplinervis* Vahl	AF, GR	Reuben et al., 2006
Botrys	*Chenopodium spp* L.	AF	Facknath, 1997
Corrosol	*Annona murricata* L.	CSI, GR	Reuben et al., 2006
L'herbe bouc, goat weed	*Ageratum conyzoides* L.	GR	Facknath, 1997
Indian privet	*Ligustrum robustum* Kaneh &Saski	IN, GR	Reuben et al., 2006
Lemon grass	*Cymbopogon citrates* DC. Stapt.	AF, GR, IN	Facknath,1997, Reuben et al., 2006
Melia, bakain, lila perse	*Melia azederach* L.	IN, AF, GR, RP,SP, CP	Reuben et al., 2006
Neem	*Azadirachta indica* L.	AF,IN,GR, RP,SP,CP, OVDT	Facknath,1996, HDRA, 2000, Reuben et al., 2006
Vetiver	*Vetivera zizanoides* L.	AF, GR	Reuben et al., 2006
Vieille fille	*Lantana camara* L.	AF, GR	Facknath, 1997
Rose	*Rosa sp*. L.	AF	Jacobson 1975 Grainge et al 1984
Legume sp.	*Calopogonium coerruleum* Benth	IN	Jacobson 1958 Grainge et al 1984
Guava	*Puniica granatum* L.	I, AF	Jacobson 1958 Grainge et al 1984
Pepper	*Pipernigrum* L.	CP	Charleston, 2004 Jacobson 1958

Source: From AMAS 1997. Food and Agricultural Research Council, Réduit, Mauritius

AF=Antifeedant, IN=insecticidal, GR= Growth regulating activity, RP= repellent, SP=Stomach poison, CSI= Chitin synthesis inhibitor, OVDT= oviposition deterrent, CP=Contact poisons.

Neem, Melia, Lemongrass, Ayapana, Vetiver and Botrys affect feeding adversely by making the treated host plant unpalatable to the pest larvae. The larvae, although remaining on the host plant, starved to death within a few days. Goat weed, Neem, Corrosol, Lemon grass, Ayapana, Vetivera, Lantana, Indian privet and Melia affect the growth and development in different ways, resulting in distorted pupae, pupal death, partial emergence of adults and deformed adults. Melia, Lemon grass and Indian privet were toxic to the larvae and caused significant mortality. Melia, however, is known to be toxic to higher animals as well, and hence its application in pest control is as yet limited. Corrosol inhibited the synthesis of chitin, an amino-polysaccharide, which together with proteins and lipids forms the insect cuticle. Lack of chitin causes death of the insect at the time of moulting and metamorphosis. Neem also repelled the adult females from laying eggs, thereby reducing the DBM population in the next generation (AMAS, 1997).

FAO (2000) reported that Neem (*A. indica*) derived from semi-arid tropical regions contains main active compounds that act as feeding deterrents and as insect growth regulators. In Ghana (Africa), Neem seed kernel extract were used against pests on several vegetable crops and had a very good effect on cabbage caterpillars probably due to the repelling action of Neem. Moreover, extracts of Neem seed kernels, applied to plutella infested cabbage plots had no adverse effects on the development and emergence of the introduced larval parasitoid of DBM, namely *Cotesia plutellae* (Hymenoptera: Braconidae).

The advantages of botanical pesticides over synthetic chemicals are by now very well-documented and well-known. Most of them are safe to prepare and apply; safe to humans, to non-target organisms, to beneficial insects, and to the environment in general; they leave no residues, hence cause neither contamination nor pollution; they are often cheaper than and just as effective as the synthetics. In most cases their bioactive compounds are fairly complex groups, thereby making it more difficult for the pest to develop resistance (FAO, 200). Birbira, *Militia ferruginia* (Hochst) Baker extracts (aqueous and chloroform) were also very effective on feeding and oviposition responses of DBM larvae (Bayeh and Brook, unpublished 2009).

Integrated Pest Management

Integrated pest management (IPM) is an approach to keeping pest populations below a level causing economic loss, through the judicious and compatible use of two or more of several possible control measures. Although definition of IPM have been proposed it is difficult to define briefly because of its inherent complexity. Perhaps the most suitable definition is that quoted by Broder (1979 cited in Fenemore and Prakash 1992). “IPM is a system that, in the context of the associated environment and the population dynamics of the pest species, utilizes all suitable techniques and methods in a compatible a manner as possible and maintains pest population at levels below those causing economic injury.” It considers the interacting factors of the environment, including the effects of adjacent crops and cultural practices, upon the crop and the environment (Fenemore and Prakash, 1992).

IPM involves using preventive control, insect monitoring and suppression tactics in a coordinated program with the purpose of managing insect population in the most efficient manner possible (Johnson, 2000). For many years, farmers depend on chemical insecticides to control DBM, but due to resistance problem of the insecticides and lack of alternative insecticides has stimulated research on the use of other control measures (Hein, 2003). Therefore, the availability of several management options including cultural, botanical and biological coupled with problem associated with insecticide use in DBM management have created a good opportunity to use an IPM approach by Brassica growers (Carl, 1992, Ttalekar and Shelton, 1993).

IPM components and methods that can be implemented for the preventive control of DBM includes: Cultural techniques such as mixed cropping of cabbage with tomato, adjustment of planting time, trap crops, avoiding use of susceptible crops and use of resistance varieties, insect growth regulators (IGRs), biological control, botanicals and use of insecticides. In IPM program, because of severe pesticide resistance problems with diamondback moth around the world, chemical insecticides should be used as little as possible to suppress pest outbreaks. For instance the report of Facknath (1997) showed that the effect of intercropping with plants of tomato, or garlic, combined with the

application of Neem seed kernel extract was found to be more efficient in protecting cabbage plants in the field.

Determination of pest damage thresholds and of economic thresholds, means of monitoring populations of pests and their natural enemies and a decision making frame work to determine action to be taken are some of the essential components of the pest management. Pest populations must be regularly monitored in IPM program to decide when to apply control measures. When certain factors are known to be important in affecting pest numbers these also must be determined. These factors may be physical, such as weather fluctuation, or biological, such as the incidence of natural enemies. Physical factors can be readily monitored by suitable instrumentation but keeping track of pests and their natural enemies requires special sampling procedures. For effective implementation of IPM monitoring of DBM should take place throughout the year. Monitoring takes place by making counts of larvae or by level of damage and by using pheromone traps (Jacobson, 2000).

An important tool for evaluating an insect problem is the economic threshold of the pest. According to stern et al. (1959) cited in Fenemore and Prakash (1992) economic threshold level is the pest population level at which economic damage begins to occur. These levels must be determined for each crop and locality, in relation to specific consumer requirements. In practice control measures almost invariably have to be initiated before pest populations reached the damage threshold level to prevent crop loss. Such a lower population level which signals the necessity for control action is referred to as the ETL. Establishment of suitable ETs is absolutely essential to the functioning of any IPM programmers (Hein, 2003).

To determine action to be taken a decision making framework is important. As IPM is essentially flexible in nature criteria for decision making as to action to be taken (such as whether to apply an insecticide or not) are of crucial importance. Some element of prediction of invents is always involved in the decision making process. For example monitoring of a field crop may indicate a moderate infestation of aphids but with a high level of parasitism. the prediction in this case may be that further significant increase in

the aphid population will not takes place and therefore spraying is not necessary (Fenemore and Prakash 1992).

The economic threshold level of this pest is determined using trap data. Control decision making is based on the action threshold. In the absence of effective level of natural enemies, appropriate control measures should be under taken, when the population of DBM, exceeds 5 larvae/10 cabbage plants. Hence, when the parasitism by *D. semiclausum* is sufficiently high, the action threshold is determined by considering rate of parasitization as follows: (Prijon, 2004).

$$y= (1-p).x$$

Where y= population of DBM larvae that can actually cause damage

p= population of DBM larvae parasitized by *D. semiclausum* (expressed as Proportion b/n 0 & 1)

x= population of 3^{rd} &4^{th} instars larvae in field, based on systematic sampling of 10 sample plants/0.2 ha of crops.

Suppression of DBM outbreak is done by the use of insecticides. However, the criteria for insecticide application include:

- If y>5 larvae/ 10 plants, then insecticide application can be undertaken.
- If y<5 larvae/ 10 plants, then insecticide application is not necessary.

3.3. Description and use of *Militia ferruginea* (Hochst) Baker

Birbira, *Militia ferruginia* (Hochst) Baker belongs to the family Fabaceae (Leguminosae), Sub-family Papilionoideae. It is a useful endemic tree species of Ethiopia with a great potential for agro-forestry (Legesse Negash, 1995). Its natural habitat is rather diverse and commonly occurs between 1000-2500m above sea level. There are two subspecies known to occur in Ethiopia. These are *M. f. ferruginea* which is confined to the northern part of the country and *M. f. darasana* which occurs in southern provinces, particularly Sidamo regions (Bekele et al., 2002). Trees from central to western Ethiopia show mixture of the two species (Azene et al., 1993: cited in Bekele et al., 2002).

Both subspecies *ferruginea* and *darasana* can grow to rather big trees, attaining the size of up to 25 m, especially when competing for light with tall moist forest trees. However, depending on the climate (particularly on the prevailing temperature and moisture regime), the two subspecies can assume the habit of a shrub or a tree. The flowers are often violet and, eventually bearing big flat pods (having the size of 27 x 3cm). The pods usually contain 5 to 10 seeds and split open along both sides to release circular and flattened seeds. During the prevalence of favorable climatic conditions, the tree may produce a large number of pods and consequently, may be considered as rather a good seeder (Legesse Negash, 1995).

Birbira is a multipurpose tree and provides the following: Its products are used for fish poisoning where mature pods and seeds are ground to fine powder and is spread over the surface of water. The fish stunned by the effect of the drug start to come close to the surface, thus enabling an easy catch. Nevertheless, there has not been any report of human death related to the consumption of Birbira intoxicated fish (Siegenthaler, 1980); the tree is extensively used as shade for coffee (Coffee arabica) in Hararge region eastern Ethiopia (Bekele et al., 2002); its pods are good source of fuel and the wood is used to make tool handles and other house hold utensils (Thulin, 1989). It is also a nice ornamental tree and is sometimes planted in towns lining streets (Amare Getahun, 1976 unpublished); Birbira is highly decomposed, its green leaves contained high

concentration of C, N and P and has a great potential as green manure for soil improvement (Fisseha Itanna et al., 2004), It is also a nitrogen fixing tree (Fassil Assefa, 1993); Birbira has also a role in the control of insect pests (Bayeh Mulatu and Tadesse G/medhin, 2000).

Chemical studies of this plant led to the isolation of several flavonoids and retenoids. Rotenone is one of the dominant compounds found in the seeds and stem barks of Birbira (Ameha Bekele, 1988). Gorge (1980) cited in Bekele et al., (2002) reported that rotenoids have been used as insecticides since 1848, when they were applied to plants to control leaf eating caterpillars. It has oral Ld_{50} of 350mg/kg and has been used as ideal general garden insecticide. It has both contact and stomach poison to insects and kill insects slowly but causes them to stop feeding almost immediately and is sold as spray concentrates and ready to use dust (Ishaaya et al., 1998).

Over 25 flavonoids, 50 isoflavonoids, 12 chalcones and miscellaneous compounds have been reported from the Militia genus alone (Amha Bekele, 1988). The crude extracts from seeds of Birbira were found to be toxic to *Sitophillus zeamais* (Bekele Jembere, 2002). In the combination with other botanicals it was found to be effective against the stem boring moth *Chilo partellus* (Sabiiti and Bekele Jembere, 2005). Together with other leaf and seed of botanicals for their insecticidal activity against the sweet potato butterfly larvae birbira out performed the other botanicals (Mesele Gemu et al., 2004).

The killing effect of Birbira extracts by contact was confirmed on three aphid species by Bayeh Mulatu (2007) through bioassay of aqueous and organic solvent extracts from the seed kernel powder, it was found that both extracts are effective in killing the aphids by contact. Daniel and Bekele (2006) assayed Birbira seed powder aqueous extract on termites under laboratory condition and recorded almost 100% mortality of different termite castes. The seed powder and oil were evaluated and found effective for the control of bean bruchids on faba bean (Bayeh Mulatu and Tadesse G/medhin, 2000).

Birbira extracts (aqueous and chloroform) were very effective on DBM larvae (Bayeh and Brook, unpublished 2009). The effectiveness of both extracts as oviposition deterrence and development retardant is very interesting scenario and needs to be

investigated further. Thus the insect was considered to study Birbira extracts that will be obtained using solvent with different polarity.

Plant parts and target insect species affect the solvent botanical extracts (Shaalan et al., 2006). In addition to this selection of solvent type has paramount importance in efficacy of botanical extractions. Shaalan et al., (2005) reviewed that solvent type for phyto-chemical extractions should be carefully selected because different solvent types can significantly affect the potency of extracted plant compounds. A reverse relationship is said to exist between extract effectiveness and solvent polarity where efficacy increases with decreasing polarity (Mulla and Su, 1999). Therefore solvents with different polarity were used to produce crude extracts from Birbira seed kernel and assayed for the efficacy against *Plutella xylostella* (L.).

4. Materials and methods

4.1. The study area

Most of the investigations in the present study were carried out at Holetta Agricultural Research Center (HARC) and partly at the Insect Science Laboratory of Addis Ababa University, Science Faculty.

4.1.2. Rearing of Test Insect

Diamondback moth, *P. xylostella* larvae and pupae were collected in cabbage farms around HARC and reared in the laboratory using 25 cm x15 cm x10 cm plastic cages at ambient temperature and humidity. Larvae and pupae were collected from the leaves using a small soft-bristle paintbrush or tear off a piece of the leaf with the larva or pupa (Rowell, 2004). Larvae were put in a rearing container and were supplied with fresh, pesticide free leaves of cabbage or other crucifer crop for food (Annex 21). The leaf petioles were trimmed with a knife or cutter. Cotton balls were soaked in plain water squeezed some of the water (but not all!) out of cotton ball and wrapped it tightly around the cut end of the leaf petiole and sealed using a rubber band. In such condition, the leaves remained alive for 2-3 days without wilting or drying out. Cages were washed, dried and changed every other day until the developed larvae pupated. The larvae were then transferred using a paintbrush to the new leaves and caged. Fresh leaves were used after 2-3 days-or whenever leaves have been eaten or have been dried out.

The pupae were transferred and kept in Petri dishes until they completed their development. The emerging adults were then transferred to a bigger cage (65 x 65 x 80 cm) (Annex 21) and maintained on distilled water and 2% sucrose solution (Odhiambo, 1999). Fresh cabbage leaves were introduced daily into this cage to provide feeding and oviposition sites.

4.1.3. Host plant

The host plants for Diamondback moth are all the crucifer crops in the cabbage family. But, for rearing and to precede the test the host plant that was selected was the Cabbage. Seeds of cabbage were bought from Addis Ababa, around Merkato. The seeds were sown

first on two larger pots in the green house. After three weeks, the seedlings were transferred singly to smaller black and medium plastic pots (Annex 21). The seedlings were watered two times a day. Sterilized soil was used for growing the seedlings. However, this was not good for the growth of the seedlings because of lack of sufficient nutrients. Compost soils were used for growing and the cabbage grew in the green house to get free from chemicals and other cabbage pests.

4.1.4. Botanical preparation and Extraction

Simple extraction method was used to obtained crude extracts from birbira seed kernel powder. Because this method is fast, simple and reproducible if it is to be performed repeatedly. This method also economical; the materials are easily available, do not used prolonged heating to drive the extraction and easily recycled by evaporation. Although, soxhlet extraction method is used widely in the extraction small to moderate volume of plant material, mainly this method is used in the extraction of fats and oils (Satyjit et al., 2006). This method uses commercially available devices for extraction and hence expensive. The other disadvantage is that it requires prolonged heating for extraction. According to Sarker et al., (2006) the main disadvantage of Soxhlet extraction method is that the extraction is constantly heated at the boiling point of the solvent used, and this can damage thermo labile compounds to form artifacts or decomposition.

Physiologically matured pods of *M. ferruginea* were collected from the premises of HARC and neighboring areas (Annex 20). The pods were then brought to the Insectary at HARC and were put aside for few days to dry. When the pod dried well, the seeds were removed from the pods. The seed coats were split to remove the cotyledons. The cotyledons were ground to powder using mortar and pestle under shady condition (Annex 20). The seed kernels were ground and the powder was kept in a sealed polythene bag in a refrigerator at 4°c until used for its crude extractions using different solvents. The powder was used to prepare different crude extracts in this experiment. All solvent extracts were prepared in the laboratory as described below:

Different polar and non-polar solvents (deionized water, acetic acid, acetone, chloroform, Toluene and hexane with the polarity index of 9.0, 6.2, 5.1, 4.1, 2.4, and 0.1,

respectively) were used for the extraction of the powder following the procedures used by Bayeh Mulatu (2007). Six samples of 50 gm of the ground seed kernels of *M. ferrugneia* were weighed separately for crude extractions with deionized water, acetic acid, acetone, chloroform, toluene and hexane. Each 50 gm sample was mixed with 100 ml solvent in separate flasks. The mixtures were stirred continuously for fifteen minutes using a magnetic stirrer at room temperature until homogenous solutions were formed and then left to stand. Each mixture was filtered using a fine cotton muslin cloth. The filtrate solutions of acetone, chloroform, toluene and hexane were transferred to evaporating dishes to evaporate the solvents using a water bath that was adjusted at the respective boiling points of each solvent in the evaporating hood. However, the deionized water and the acetic acid were evaporated using rotary evaporator under vacuum, in a boiling bath. After evaporating the solvents, solid extracts were left on each container. The solid extracts were transferred separately to flat polythene and kept spread for a day in a ventilated room for further removal of the solvents that might have remained in the solid extracts. All the extracts were packed in polythene bags and kept in a refrigerator at 4^0C to maintain their freshness until used for the assays.

The solid extracts were used to prepare the serial dilutions. Each serial concentration (0.2, 0.4, 0.6, 0.8, 1.0, 1.2, and 1.4mg/ml) were prepared by dissolving 20 ,40 ,60 ,80 ,100 ,120 and 140 mg of birbira powder with 100 ml of water. The solution was thoroughly mixed with a glass rod and stirred by a magnetic stirrer until lemonade solution was formed. Water was used as application solvent and the solution was ready for application on the cabbage leaves to bioassay their effects on DBM eggs, larvae or adults. Similarly, the extraction was done for all the solvents that used.

4.1.5. Feeding deterrence bioassay

This assay was conducted using excised leaves of cabbage seedlings; it was taken from seedlings that were raised for the purpose in completely pesticide free environment. The working lab bench and all the Petri dishes were disinfested by 150 ml of ethanol diluted with 50 ml of distilled water. The test leaves were washed to remove dust. Then all the washed leaves were put on blot papers to remove the surface water. Dilutions of all the six extracts from birbira seed kernel powder were poured into separate bowls and kept at

room temperature for 1hr before use. Leaf dipping method was used to run the feeding bioassay (Trisyono and Whalon, 1999: cited in Bayeh Mulatu, 2007) using the cabbage leaves.

The test leaves were dipped for 1 hr in the dilutions prepared at (w/v): 0, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2 and 1.4 mg/ml. They were transferred into separate Petri dishes immediately after the excess liquid was dislodged from them. The cut end of each treated leaf was covered with tap water wetted cotton ball. Then after five 3rd instars larvae of DBM that were starved for three hours a priory were introduced into each Petri dish. All Petri dishes were maintained in the HARC insectary at ambient temperature and humidity by putting the Petri dishes in a completely randomized design on a lab bench (Annex 25) below. Follow-ups were made on their feeding activity until the last larvae in each Petri dish either died or pupated. The contents were treated with solvents. Every day, data were collected on the number of larvae alive and feeding and pupating and days to pupation. A total of 40 replications on a total of 200 larvae per rate were run for each extract.

4.1.6. Oviposition deterrence bioassay

Similarly, this assay was carried out using excised leaves of cabbage. The test leaves were undergo similar treatments as described above. The Oviposition arena were different where leaves treated with birbira extract at one of the eight concentrations (0, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, and 1.4 mg/ml) prepared in the bowls. Leaves were dipped for 1hr, dislodge the liquid, dried and the cut end of each treated leaf was covered with tap water wetted cotton ball. Treated leaves were put into separate Petri dishes, and transferred into an oviposition cage and were arranged in a completely randomized way within the cage (Annex 25). The Petri dishes were left open in the cages and three pairs of newly emerged naive adults of DBM were introduce

They were provided with 10% sugar solution soaked in cotton balls. Totally 40 replications on a total of 48 adults were run per treatment. The experiment was carried out in the HARC insectary at ambient temperature and humidity by putting the cages in a completely randomized design. All the cages were covered with cloth to produce dark condition for the adults. The introduced adults were allowed to mate and oviposit for

three days and all the adults were removed afterwards. The number of laid eggs were checked and counted on leaves, in the Petri dishes walls and in the inner surface of the cages after 72 hrs.

4.1.7. Ovicidal bioassay

In this assay 40 fresh untreated excised cabbage leaves were taken from the greenhouse, the cut end of each treated leaf were covered with tap water wetted cotton ball and five pairs of newly emerged naive adults of DBM were introduced in a bigger cage (Annex 24). In the cages adults were provided 10 % sugar solution soaked in cotton balls and allowed to mate and oviposit. Mating is expected to occur soon after emergence from the cocoon. And all the adults were removed afterwards after the oviposition period was over (i.e. after 72 hrs). Eggs that were laid on each untreated fresh cabbage leaves in each mica cages were counted and recorded.

Eggs that were counted up to twenty five on each untreated leaf recorded and labeled on pieces of paper with pencil and pinned to the leaf stalk with small pins. Then leaves which contain 25 eggs for each concentration were dipped for 30 sec. into each extracts of the serial concentrations (0, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, and 1.4 mg/ml) prepared in the bowls. After 30 sec. eggs that received same treatment were taken out, dried on paper bowel, placed into each transparent square plastic cage and kept at room conditions until any egg hatchability is completed or died (Fig 2).

4.1.8. Larval settlement bioassay

In the larval settlement bioassay fresh untreated excised cabbage leaves were taken from the greenhouse, washed with water to remove the dust and dipped into the prepared bowels containing each concentration of the sample (including crude and control) for 1hr, making sure that both sides were thoroughly wetted, and the leaves were hung up to dry and dislodge the fluid. Once dry, the cut end of each treated leaf was covered with tap water wetted cotton ball. After this each treated leaf was placed in labeled Petri dishes used for each treatment. After the cabbage leaves were set in such a way, five neonate larvae of DBM that were starved for 3hrs were transferred into each Petri dish.

All Petri dishes were maintained in an in sectary at ambient temperature and humidity by putting the Petri dishes in a completely randomized design on a lab bench (Fig 1). A total of 5 replications on a total of 25 larvae per rate were run for each extract. Larvae may die while attempting to settle and this were also recorded. The test were done by transferring newly emerging larvae five per leaf and record the time it takes for larvae to settle and feed under the leaf sheath after 24hr.

4.1.9. Data Analysis

All the experiments were laid in a completely randomized design (CRD). For the data on feeding responses of DBM to all the extracts of Birbira seed kernel powder one way ANOVA (JMP In, 2003) were used on the corrected percent mortality data percent mortality, days to highest and total larval mortality, number of larvae that pupated and days to pupation, days of adult emergence, number of adults emerged and the appearance of emerging adults. Mean comparisons were conducted using Tukey Kramer honest significance test, and P value was set at 5% probability level. On the oviposition response, ovicidal and larval settlement effect data, one way ANOVA were used on the number of eggs laid on leaves and on the Petri dishes, number of eggs oviposited per leaf, eggs hatchability or died and on the number of settled larval per a leaf.

Probit analysis (SPSS 10 for windows, 2003) was made to produce the dosage-mortality curves for all the applications of different solvent extracts. The LC_{50} values and the susceptibility of a DBM egg and larvae to a treatment of each serial concentration and solvent extracts were assessed by constructing a concentration mortality curve in which the concentration is plotted against the percentage mortality at a given time. Mortality for eggs and larvae in the control treatment was used to correct mortality in the other treatments. This adjustment was obtained using Abottt's formula (Abbott 1925, cited in Ishaaya et al., 1998; Sharaby, 1988) as follows.

$$\% \text{ corrected mortality} = \frac{P - Po}{100 - Po} \times 100$$

Where P = % mortality in the control of treated insects

Po = % mortality of insects in the untreated control

Using Probit regression (SPSS 13.0) the LC_{50} values for the different extracts were determined. Dose dependent egg and larval mortality curves were produced using the Probit outputs.

5. Result

5.1. Feeding Response of DBM to Birbira extract

Mean percent total larval mortality in birbira treated leaves was significantly affected by the interaction of the extracts. Birbira extract obtained using Acetic acid caused rate dependent mortality of DBM larvae. The highest mortality was recorded at 0.8 and 1.2 mg/ml, which were significantly different from the lower rates and the control ($F_{6, 27}$ =13.2, P<0.0001) (Annex 1). The mortality at 1.4 mg/ml was not different statistically from 1.2 mg/ml. Acetone extract also caused significant larval mortality at higher rates of extract application ($F_{6,27}$ =23.0, P<0.0001). Chloroform extract caused the highest mortality at the rate1.2 mg/ml. Toluene extract caused significantly lower larval mortality at the lower rates of application ($F_{6,27}$ =7.6, P<0.0001). At 1.2 mg/ml Chloroform extract caused the highest larval mortality than the other rates. At 1.0 mg/ml Hexane caused the highest larval mortality than the other rates.

At 0.2 mg/ml Water caused the highest larval mortality than the other extracts ($F_{5,24}$ =8.7, P<0.0001) (Annex 12). At 0.2 and 0.4 mg/ml, least mortality was recorded by Toluene extract. At 0.8 and 1.2 mg/ml Acetic acid caused the highest larval mortality than the other extracts. Acetone extract also caused significant larval mortality at higher rates of extract application. The highest larval mortality recorded for Hexane extract was at 1.0 mg/ml.

Table 1. Mean (±SE) Percent larval mortality of *P. xylostella* larvae on cabbage leaves treated with different solvent extracts of birbira seeds powder

Rate	Water	Acetic acid	Acetone	Chloroform	Toluene	Hexane
0	19.0±7.48Ad	22.0±4.01Aad	28.0±8.00Bc	16.2±6.45Cd	16.0±6.40Cc	22.0±10.00Ba
0.2	89.28± 6.50Abc	30.36± 6.56Dd	46.43±0.00Cc	46.43±11.90Cac	7.8±10.02Bac	25.00±19.68Ca
0.4	94.64±5.40Adc	62.50±13.65Cb	83.92±6.56Ad	67.85±13.12Cc	19.64±11.97Dc	67.85±13.12Cc
0.6	100.00±0.00Ab	67.85± 5.35Bb	78.572±5.35Bbd	41.07±10.00Cb	14.4±12.80Dabc	67.85±13.12Bc
0.8	100.00±0.00Ab	100.00± 0.00Aa	86.60±7.73Ad	66.519±6.69Bc	67.85±15.61Cd	78.57±10.02Cc
1.0	94.64±5.35Ac	89.28± 6.56Aa	100.00±0.00Aa	78.57±5.35Bc	73.21±11.97Bd	83.92±10.71Ac
1.2	94.64±5.35Aa	100.0± 0.00Aa	100.00±0.00Aa	93.30±6.69Aa	57.14±13.60Bd	78.57±13.12Ac
1.4	100.00±0.00Aa	94.64± 5.35Aa	100.00±0.00Aa	89.28±6.56Aa	73.21±8.47Bd	83.92± 6.56Ac

Means followed by the same letter (lowercase) within a column and (uppercase) within a row are not significantly different at P=0.05 (Tukey-Kramer HSD, JMP In 2003).

Days to first larval mortality

Days to the 1st larval mortality was observed within one day after the exposure of the 3rd instar larvae on leaves that were treated with the different solvent extracts of birbira, except that of the water extract. The shortest time to larval mortality were recorded on WA extracted birbira was applied at 1.4 mg/ml and was not significantly different from the other higher rates ($F_{7, 32=}$1.7, P>0.12) (Annex 3). The other extracts with shorter days to larval mortality brought the effect latter than water extract were AC and CH. Days to the 1st larval mortality caused by these extracts were significantly higher at the rate of 1.0 and 1.2 mg/ml, respectively. Mortality on this day was significantly lower for TO and HE extracts even at the higher rates of application (Table 2).

The between treatment comparison clearly showed that at 0.2 mg/ml the days to 1st highest larval mortality was found within one day after the exposure of AA and TO extract than the others. This is also the case for HE at 0.4 mg/ml. At 0.6 mg/ml significantly longer days to larval mortality was found on AA and longest on HE than the other extracts ($F_{5,24}$ =10.0, P<0.0001) (Annex 14). At 0.8 mg/ml longest days to larval mortality was observed for AC but this was not significantly different from the others

(P>0.438). At 1.0 mg/ml AC shows longest days to larval mortality and at 1.2 mg/ml the longest days mortality was found on CH.

Table 2. Mean (±SE) days to first larval mortality on cabbage leaves treated with different solvent extracts of birbira seed powder

Rate	Mean ±SE days to first mortality					
	Water	Acetic acid	Acetone	Chloroform	Toluene	Hexane
0	0.00±0.00Ab	0.00±0.00Aa	0.20±0.20Ba	0.00±0.00Aa	0.00±0.00Ab	0.00±0.00Aa
0.2	0.00±0.00Bb	0.40±0.40Aa	0.20±0.20Ca	0.20±0.20Ca	0.40±0.40Ab	0.00±0.00Ba
0.4	0.00±0.00Ab	0.20±0.20Ba	0.00±0.00Ac	0.20±0.20Ba	0.60±0.40Cb	0.60±0.24Cb
0.6	0.00±0.00Bb	0.40±0.40Aa	0.20±0.20Ba	0.00±0.00Ba	0.00±0.00Bb	1.00±0.32Cc
0.8	0.00±0.00Ab	0.40±0.24Ba	0.80±0.37Bc	0.20±0.20Ba	0.60±0.60Bb	0.20±0.20Bb
1.0	0.40±0.24Cb	0.40±0.24Ca	1.60±0.67Ab	0.20±0.20Ca	0.40±0.40CAb	0.00±0.00Da
1.2	0.40±0.24Bb	0.60±0.40Ba	0.60±0.24Bc	1.00±0.44Aa	0.00±0.00Bb	0.40±0.40Bb
1.4	0.60±0.40Ab	0.60±0.40Aa	0.40±0.24Ac	0.40±0.24Aa	0.00±0.00Ab	0.00±0.00Aa

Means followed by the same letter (lowercase) within a column and (uppercase) across a row are not significantly different at P=0.05 (Tukey-Kramer HSD, JMP In 2003).

Days to last larval mortality

Days to the last larval mortality was also significantly affected by the interaction of the extracts. The laboratory experiment clearly showed that, last DBM larvae mortality after fed on treated leaves with all solvent extracts of birbira were observed on the 14th day after treatment application. In all the solvent extracts larval mortality significantly increased with increasing rates of application (Table 3).

The between treatment comparison showed that there was a significant shorter days of mortality at 0.2 mg/ml for WA extract. The day at which significantly high larval mortality was recorded was at 1.4 mg/ml for WA. The day at which the last larval mortality was significantly high was at 0.4 and 0.6 mg/ml for WA and least for TO extract. There was no significant difference in the days to highest number of last larval mortality at 1.0, 1.2 and 1.4 mg/ml for AC, AA and WA extract, respectively ($F_{5,24}$ =1.7, P>0.17) (Annex 14).

Table 3. Mean (±SE) days to last larval mortality on cabbage leaves treated with different solvent extracts of birbira seed powder

Rate	Water	Acetic acid	Acetone	Chloroform	Toluene	Hexane
0	11.00±0.31Ac	11.40±0.24Aa	11.60±0.50Aa	10.80±0.37Aa	10.80±0.4Aac	12.00±0.31Aa
0.2	14.80±0.20Ab	12.40±0.24Bbc	13.00±0.00Ba	13.00±0.44Bc	11.20±0.37Cb	12.20±0.7Bcb
0.4	14.80±0.20Ab	13.60±0.50Bc	14.40±0.24Bb	13.80±0.48Bc	12.00±0.44Cc	13.80±0.48Bb
0.6	15.00±0.00Ab	13.80±0.20Bc	14.20±0.20Bb	12.80±0.37Bb	12.00±0.83Cc	13.80±0.48Ab
0.8	14.60±0.40Ab	14.20±0.80Ac	14.20±0.37Ab	13.40±0.40Ac	13.80±0.58Ad	14.20±0.37Ab
1.0	14.80±0.20Bb	14.60±0.24Ad	15.00±0.00Bb	14.20±0.20Ac	14.00±0.44Ad	14.40±0.4Abd
1.2	14.60±0.24Bb	15.00±0.00Ad	15.00±0.00Ab	14.40±0.40Bc	13.40±0.5Bdb	14.20±0.48Bb
1.4	15.00±0.00Ab	14.80±0.20Ad	15.00±0.00Ab	14.60±0.24Bcb	14.00±0.31Bd	14.40±0.2Bbd

Means followed by the same letter (lowercase) within a column and (uppercase) within a row are not Significantly different at P=0.05 (Tukey-Kramer HSD, JMP In 2003).

Days to highest larval mortality

Days to highest larval mortality was significantly affected by the interaction of the extracts. For the WA, extract the number of days to the highest larval mortality was significantly longer at 0.8 mg/ml rates of application ($P<0.002$) and shorter at the highest rate 1.4 mg/ml, and the control where natural mortality is known to take place at a certain time in the course of the experiment. The other rates took about 8 days to cause same effect.

For AA extract days to the highest larval mortality was shorter at 0.6 and 1.0 mg/ml and no significant longer period of highest larval mortality for the other rates ($F_{7, 32} =1.7$, $P>0.1$). The day to highest larval mortality for AC extract was significantly shorter at the 0.4 and 0.8 mg/ml rates of application ($F_{7, 32} =9.5$, $P<0.0001$) (Annex 2). For CH extract days to the highest larval mortality was significantly longer at 0.6 and 1.4 mg/ml ($F_{7, 32} =3.2$, $P<0.01$). The Day to the highest larval mortality was longer at 1.2 mg/ml for TO extract. There was no significant difference for the longer periods of HE extract at 0.4 mg/ml and no significant longer period of highest larval mortality for the other ($F_{7, 32} =1.8$, $P>0.10$).

The between treatment comparison show that at 0.2 mg/ml the days to the highest larval mortality was found on CH and is significantly different from the other extracts ($P<0.004$). At 0.4 mg/ml AC caused significant days to the highest larval mortality than the other extracts ($P<0.000$). The days to the highest larval mortality was found to be at 0.6 mg/ml for AA than the other extracts and this is not significantly different from the other extracts ($F_{5,24} = 7.8$, $P>0.141$) (Annex 13). At o.8 mg/ml days to the highest larva mortality was shorter for AA and no significant longer period of highest larval mortality for the other extracts ($P>0.39$). At 1.0 mg/ml days to the highest larval mortality was shorter for AC and no significant longer period of highest larval mortality for the other extracts ($P>0.09$). At 1.2 mg/ml days to the highest larval mortality was longer for TO and at 1.4 mg/ml for CH than the other extracts (Table 4).

Table 4. Mean (±SE) days to highest larval mortality on cabbage leaves treated with different solvent extracts of birbira seed powder

Rate	Water	Acetic acid	Acetone	Chloroform	Toluene	Hexane
0	5.40±1.8Aad	6.80±1.39Aa	3.20±1.06Ba	3.40±1.46Ba	4.20±1.8Bad	6.60±1.32Aa
0.2	7.20±0.96Bd	9.80±1.52Aa	8.80±0.58Bb	11.0±1.14Ab	4.20±1.4Bbd	7.80±0.58Ba
0.4	4.00±0.5Cbd	8.80±1.71Ba	12.20±0.9Ab	9.80±1.82Bb	5.8±0.96Ccd	12.0±1.1ABa
0.6	8.00±0.94Bd	12.00±0.6Aa	11.00±1.3Ab	11.40±1.1Ab	7.80±2.10Bd	11.60±1.7Aa
0.8	11.20±1.01Ae	8.40±1.63Ba	12.00±1.04Ab	9.40±1.46Bb	8.40±1.80Bd	11.20±1.82Aa
1.0	10.20±0.9Ae	11.40±0.9Ca	7.40±0.7Bab	10.00±1.09Ab	11.80±1.1Ce	10.20±1.5Aa
1.2	8.20±1.3Cd	8.00±1.30Ca	7.20±0.66Cac	9.80±1.82Bb	12.80±1.0Ae	9.80±1.15Ba
1.4	5.60±1.7Acd	10.20±1.2Ba	8.80±1.2Bb	11.20±1.3Cb	9.80±1.2Bd	10.80±1.4Ba

Means followed by the same letter (lowercase) within a column and (uppercase) within a row are not significantly different at P=0.05 (Tukey-Kramer HSD, JMP in 2003).

Days to pupation & larvae pupated on the first date

Pupation was also significantly affected by the interaction of the extracts. There was a significant difference in the number of days it took for the larvae to pupate after fed on leaves that were treated with different solvent extracts of birbira. Larvae that were developed into pupae in the control treated leaves took 8 days to pupate. However, no larvae were pupated at this day at all the rates for WA, AC, CH and HE extracts and only at the higher rates for AA and TO extracts (Table 7). The days to highest pupation had significantly increased as the rates of extract application increased for all the treatments except for WA, AA and AC at the higher rates. This was also the case for days to last pupation except for WA and AC extracts at 1.4 mg/ml.

The within treatment comparisons of the mean number larvae pupated shows that for WA and AC extract no larvae managed to pupate after 8 days. Pupation occurred after 10 days, but the number pupated was very low. In the control, most of the larvae pupated on 8 days and the rest on the 9^{th} days. For AA extract no larvae managed to pupate after 7 days at the higher rates. Pupation occurred after 9 days, but the number of pupated was very low. In the control, most of the larvae pupated on 7 days. For CH extract, no larvae managed to pupate after 7 days. Pupation occurred after 9 days, but the number pupated was very low. For TO extract pupation occurred on 8 days at all the rates except 0.8 and 1.4 mg/ml, however, the number of larvae pupated decreases as the rate increased as compared to the control. For HE extract no larvae managed to pupate after 7 days. Pupation occurred after 9 days, but the number pupated was very low. In the control most of the larvae pupated on 8 days and the rest on the 9^{th} days (Table 6).

Table 6. Days to pupation of *P. xylostella* larvae on cabbage leaves treated with different solvent extracts of birbira seed powder.

Rate	Treatments								
	Water			Acetic acid			Acetone		
	DFP	DHP	DLP	DFP	DHP	DLP	DFP	DHP	DLP
0	8(10)	9(7)	11(1)	7(9)	7(9)	10(2)	8(10)	8(10)	10(2)
0.2	-	10(1)	10(1)	7(1)	8(5)	13(1)	-	10(5)	14(1)
0.4	-	10(1)	10(1)	7(1)	9(2)	11(1)	-	10(1)	13(1)
0.6	-	-	-	7(1)	13(2)	13(2)	-	14(2)	14(2)
0.8	-	10(1)	14(1)	-	13(2)	13(2)	-	14(2)	14(2)
1.0	-	10(1)	10(1)	-	10(1)	12(1)	-	-	-
1.2	-	9(1)	12(1)	-	-	-	-	-	-
1.4	-	-	-	-	13(1)	13(1)	-	-	-

Rate	Treatments								
	Chloroform			Toluene			Hexane		
	DFP	DHP	DLP	DFP	DHP	DLP	DFP	DHP	DLP
0	7(5)	8(10)	10(1)	8(15)	8(15)	10(4)	7(5)	7(5)	10(4)
0.2	-	11(3)	14(2)	8(6)	9(7)	12(3)	-	10(5)	14(1)
0.4	-	9(2)	14(2)	8(9)	8(5)	13(1)	-	11(2)	15(1)
0.6	-	11(5)	14(2)	8(6)	8(6)	12(3)	-	9(2)	13(1)
0.8	-	9(3)	14(1)	-	10(4)	13(1)	-	9(1)	15(1)
1.0	-	11(2)	11(2)	8(2)	8(2)	10(2)	-	11(2)	12(1)
1.2	-	11(1)	14(1)	8(4)	10(3)	10(3)	-	9(3)	13(1)
1.4	-	11(1)	15(1)	-	10(4)	10(4)	-	10(1)	13(1)

- Note: Numbers in parenthesis indicates number larvae pupated.
  DFP=days to first pupation; DHP = days to highest pupation; DLP =days to last pupation

Table 7. Mean (±SE) pupation and number of *P. xylostella* larvae that managed to pupate on the 1st day by rate on cabbage leaves treated with different extracts of birbira seed powder

Rates	Water	Acetic acid	Acetone	Chloroform	Toluene	Hexane
0	2.00±0.547Aa	1.60±0.24Aa	2.00±0.31Aa	1.00±0.54Ba	3.00±0.31Ca	1.00±0.54Ba
0.2	0.00±0.00Ab	0.20±0.20Bb	0.00±0.00Ab	0.00±0.00Ab	1.20±0.80Ba	0.00±0.00Aba
0.4	0.00±0.00Bc	0.20±0.20ABb	0.00±0.00Bb	0.00±0.00Bb	1.80±0.91Aa	0.00±0.00Bba
0.6	0.00±0.00Ad	0.20±0.20Bb	0.00±0.00Ab	0.00±0.00Ab	1.20±0.37Ba	0.00±0.00Aba
0.8	0.00±0.00Aab	0.00±0.00Ab	0.00±0.00Ab	0.00±0.00Ab	0.00±0.00Ab	0.00±0.20Aa
1.0	0.00±0.00Aac	0.00±0.00Ab	0.00±0.00Ab	0.00±0.00Ab	0.40±0.40Ac	0.00±0.00Aba
1.2	0.00±0.00Abc	0.00±0.00Ab	0.00±0.00Ab	0.00±0.00Ab	0.80±0.20Ba	0.00±0.00bAa
1.4	0.00±0.00Abd	0.00±0.00Ab	0.00±0.00Ab	0.00±0.00Ab	0.00±0.00Ad	0.00±0.00Aba

Means followed by the same letter (lowercase) within a column and (uppercase) within a row are not significantly different at P=0.05 (Tukey-Kramer HSD, JMP In 2003).

Highest number of larvae pupated in a day

There was significant difference for the highest number of larvae pupated on leaves that were fed on treated leaves with different solvent extracts of birbira. The within treatment comparison between the rates show a clear pattern in which more larvae managed to pupate on the untreated leaves and leaves treated with the different extracts at lower rates. The extract with WA allowed very few larvae to pupate at 1.0 mg/ml where as AA extract allowed fewer larvae to pupate at the rate 1.4 mg/ml. The AC extract prevented pupation at the rate between 0.8 and 1.4 mg/ml. CH and HE extracts also allowed fewer larvae to pupate at the higher rates. In contrast more larvae pupated in TO extract at all the rates (Table 8).

The between treatment comparison clearly show that significantly least number of larvae were pupated at 0.2 mg/ml for WA extract than the others. Significantly high numbers of larvae were pupated at 0.4 mg/ml for TO and no pupation was recorded at 0.6 mg/ml for WA extract. This was also the case for AC at 0.8 mg/ml and for AA at 1.2 mg/ml, but this was not significant to the other extracts ($F_{5,23}$ =2.2, P>0.07) (Annex 16). There was no pupation between the rates 0.8 and 1.4 mg/ml for AC extract. The highest number of

larvae pupated at 1.4 mg/ml for TO extract was not significantly different to the other extracts (P>0.23) (Table 8).

Table 8. Mean (±SE) highest number of larvae pupated in a day on cabbage leaves treated with different solvent extracts of birbira seed powder

Rates	Water	Acetic acid	Acetone	Chloroform	Toluene	Hexane
0	2.60±0.24Aa	1.80±0.20Aa	2.40±0.24Aa	2.80±0.20Ba	3.20±0.20Ba	2.60±0.24Aa
0.2	0.20±0.20Ac	1.60±0.24Ba	1.60±0.24Bb	1.60±0.24Bb	2.60±0.40Ca	1.60±0.40Ba
0.4	0.20±0.20Ad	0.60±0.24Ab	0.60±0.24Ad	1.40±0.24Cd	2.40±0.67Ba	\ 0.60±0.24Ac
0.6	0.00±0.00Cab	1.00±0.00Aa	0.80±0.20Ac	1.40±0.24Bc	1.80±0.48Ba	1.00±0.31Ab
0.8	0.40±0.24Bbc	0.40±0.40Bc	0.00±0.00Aab	0.60±0.24Bab	0.80±0.37Bd	0.60±0.24Bd
1.0	0.20±0.20Ab	0.40±0.24Ad	0.00±0.00Bac	0.40±0.24Aac	1.00±0.44Cc	0.60±0.40Aac
1.2	0.40±0.24Bbd	0.00±0.00Aab	0.00±0.00Abc	0.40±0.24Bbc	1.00±0.31Cb	0.60±0.40Bab
1.4	0.00±0.0Aac	0.20±0.20Bac	0.00±0.00Abd	0.40±0.24Bcd	0.80±0.20Bab	0.60±0.24Bbc

Means followed by same letter (lowercase) within a column and (uppercase) within a row are not significantly different at P=0.05 (Tukey-Kramer HSD, JMP In 2003).

Total larvae pupated

Pupation was also significantly affected by the interaction of the extracts. There was a significant difference in the total number of larvae that developed into pupae between larvae that were fed on leaves treated with the six solvent extracts.

The within treatment comparison between the rates shows that the total number of larvae that developed into pupae was found higher on the untreated leaves and leaves treated with the different extracts at lower rates. For WA extract, the total number of larvae pupated was lower at the rates between 0.4 and 1.0 mg/ml and no pupation was recorded at 0.6 and 1.4 mg/ml. For AA extract the total number of larvae pupated were significantly higher at 0.2 mg/ml and lower at the rate 1.4 mg/ml and no pupation was recorded at 1.2 mg/ml ($F_{7, 32}$ =8.9, P<0.0001) (Annex 7). For AC extract there was no larvae pupated at all the higher rates of application. The total number of larvae pupated at the day generally decreases as the rates increases for CH, TO and HE extracts (Table 9).

The between treatments comparison at each rate shows that significantly higher number of larvae pupated in TO extract at the rates (0.2- a0.6 mg/ml) treated leaves and the least number pupated in the WA extract treated leaves ($F_{5, 24,}$ =6.8, P<0.0004). No larvae were pupated at 1.0 mg/ml for AC at 1.2 mg/ml for AA than the other extracts. There was no significant difference in the highest numbers of larvae that were pupated at 0.8 mg/ml for CH extract ($F_{5, 24,}$ =0.5, P>0.76) (Annex 18). There was a significant difference for the higher numbers of larvae pupated at 1.2 mg/ml for TO and HE extracts than the others (Table 9).

Table 9. Mean (±SE) total larvae pupated on cabbage leaves treated with different solvent extracts of birbira seed powder

Rates	Water	Acetic acid	Acetone	Chloroform	Toluene	Hexane
0	4.00±0.31Aa	3.60±0.24Ba	3.40±0.50Bd	4.00±0.44Ac	4.20±0.37Aa	3.20±0.37Bc
0.2	0.20±0.20Ab	2.60±0.50Ba	2.00±0.00Bad	2.40±0.50Bc	3.8±0.37Ca	2.80±0.73Bc
0.4	0.20±0.20Cc	1.00±0.54Ab	0.60±0.24Aab	1.00±0.44Aa	3.0±0.44Ba	1.20±0.4Ab
0.6	0.00±0.00Ad	1.20±0.20Bc	1.00±0.31Bac	2.00±0.31Cb	3.0±0.83Ca	1.40±0.4Bb
0.8	0.60±0.40Aab	0.80±0.80Ad	0.80±0.37Abc	1.60±0.40Bd	1.2±0.58Bb	0.80±0.37Aa
1.0	0.20±0.20Dac	0.40±0.24Bab	0.00±0.00Ac	0.40±0.24Bab	1.0±0.44Cc	0.60±0.40Bd
1.2	0.40±0.24Cbc	0.00±0.00Aac	0.00±0.00Ab	0.40±0.24Cac	1.6±0.50Bab	1.00±0.63Bab
1.4	0.00±0.00Acd	0.20±0.20Aad	0.00±0.00Aa	0.60±0.40Aad	0.8±0.20Ad	0.60±0.24Aac

Means followed by the same letter (lowercase) within a column and (uppercase) within a row are not significantly different at P=0.05 (Tukey-Kramer HSD, JMP In 2003).

Adult emergence

There was a significant difference in larvae that were fed on treated leaves with solvent extracts of birbira that survived into adult moth stage. The within treatment comparison between the rates shows that significantly higher numbers of larvae were developed into a moth stage at the rate 0.8 mg/ml in the WA extract and no larvae were developed into a moth stage for the rates 0.6 and 1.4 mg/ml ($F_{7, 32}$ =33.5, P<0.0001) (Annex 8). The highest percentage of adult emerged were found on the AA extract at the rate 0.2 mg/ml than the others and the lowest percentage of adult emerged were at 1.4 mg/ml, no adults were emerged at 1.2 mg/ml. Significantly higher numbers of larvae were developed into

adult moth stage at the rate 0.2 mg/ml for AC and there were no adults emerged at all the higher rates ($F_{7, 32}$ =30.6, P<0.0001). The highest and the lowest percentage of adult emerged were also found at the rate 0.2 and 1.0 mg/ml for CH and at 0.2 and 1.4 mg/ml for TO and HE extracts (Table 10).

The between treatments comparison at each rate shows that the highest percentage of adult emerged was found at 0.2 mg/ml for TO extracts than the other extracts. The lowest percentage of adult emerged was found for WA extracted birbira at 0.4 mg/ml, and no adults were emerged at 0.6 mg/ml for this extract. There was no significant difference for the highest percentage of adult emerged at 0.8 mg/ml for CH extract ($F_{5, 24}$ =0.9, P>0.44) (Annex 19). For AC extract no adult moths were emerged for all the higher rates of application. And this was also the case for AA at 1.2 mg/ml than the other extracts (Table 10).

Table 10. (Mean ±SE) percent adult emerged of P. xylostella on treated cabbage leaves treated with different solvent extracts of birbira seed powder

Rate	Water	Acetic acid	Acetone	Chloroform	Toluene	Hexane
0	84.0±7.48Aa	72.0± 4.89Ba	72.00±8.0Bbc	84.0± 7.48Aa	84.0± 7.48Aa	68.0± 4.89Ba
0.2	4.00±4.0Db	52.0±10.19Ba	40.0±6.32Cb	40.0±14.14Cb	80.0± 6.32Aa	56.0±19.39Ba
0.4	4.00±4.0Cb	8.0± 4.89Bc	8.0±4.89Bab	12.0± 4.89Bc	60.0± 8.94Ab	24.0± 9.79Dc
0.6	0.00±0.0Aab	20.0± 0.0Cb	16.0±4.00Bac	32.0±10.19Cab	56.0±17.20Cb	28.0± 8.00Cc
0.8	12.00±8.0Abc	8.0± 8.0Ad	12.0±4.89Aad	28.0± 4.89Aac	24.0±11.66Ac	16.0± 7.48Ad
1.0	4.00±4.0Bb	8.0± 4.89Bab	0.0±0.00Bc	4.0± 4.00Bd	20.0± 8.9Aab	12.0± 8.00Bab
1.2	8.0±4.48Ccd	0.00± 0.0Aac	0.0±0.00Bd	8.0± 4.89Cbc	32.0±10.19Cd	20.0±12.64Cb
1.4	0.00±0.0Aac	4.00± 4.0Bad	0.0±0.00Ca	8.0± 8.00Bcd	16.0± 4.00Bbc	12.0± 4.89Bab

Means followed by the same letter (lowercase) within a column and (uppercase) within a row are not significantly different at P=0.05 (Tukey-Kramer HSD, JMP In 2003).

Days to adult emergence

There was a significant difference in the days to the larvae to emerge or develop to adult moth stage after feeding on leaves that were treated with different solvent extracts of birbira. The within treatment comparisons between the rates show that for WA extract adult emergence occurred after 17 days, but the number of adult emerged was very low. In the control most of the adults emerged on 14 day. For AA extract adults emerged after 17 days but the number adult emerged were very low. For AC extract no adults were emerged after 14 day at the higher rates. For CH extract adults emergence occurred after 16 days but the number of adult emerged decreased as rates increases. For TO extract the days to adult emerged were on the 17 days and for HE were after 18 days (Table 11).

The between treatments comparison at each rate shows that at 0.2 and 0.4 mg/ml the longer period (days) to adult emergence were recorded for HE extract than the others. At 0.6 mg/ml it was a day where no adults emerged for WA extract and this was also the case for AC at all the higher rates of applications. At 0.8 mg/ml the longer period (days) to adult emergence were recorded for TO extract than the others. At 1.4 mg/ml the longer period (days) to adult emergence were recorded for TO, however no adults were emerged at these days for WA extracts (Table 11).

Table 11. Days to adult emergence of *P. xylostella* on cabbage leaves treated with different solvent extracts of birbira seed powder

Rate	Treatments								
	Water			Acetic acid			Acetone		
	DFAE	DHAE	DLAE	DFAE	DHAE	DLAE	DFAE	DHAE	DLAE
0	14(13)	14(13)	17(1)	14(4)	14(4)	19(2)	14(6)	17(7)	17(7)
0.2	-	17(1)	17(1)	-	17(6)	19(3)	-	17(7)	18(3)
0.4	-	18(1)	18(1)	-	16(1)	19(1)	-	17(1)	17(1)
0.6	-	-	-	-	17(3)	18(2)	-	17(3)	19(1)
0.8	-	17(1)	21(1)	-	17(2)	17(2)	-	17(3)	17(3)
1.0	-	17(1)	17(1)	-	17(1)	19(1)	-	-	-
1.2	-	21(2)	21(2)	-	-	-	-	-	-
1.4	-	-	-	-	19(1)	19(1)	-	-	-

Rate	Treatments								
	Chloroform			Toluene			Hexane		
	DFAE	DHAE	DLAE	DFAE	DHAE	DLAE	DFAE	DHAE	DLAE
0	14(4)	16(9)	17(2)	14(10)	14(10)	17(1)	14(1)	18(9)	18(8)
0.2	-	16(8)	16(4)	14(1)	16(13)	20(3)	-	18(9)	18(9)
0.4	-	17(2)	17(2)	-	18(5)	21(1)	-	16(2)	20(2)
0.6	-	17(6)	17(6)	-	17(6)	21(2)	-	18(3)	19(2)
0.8	-	16(5)	17(5)	-	21(3)	21(3)	-	16(1)	20(1)
1.0	-	17(1)	17(1)	-	19(3)	21(1)	-	18(1)	20(1)
1.2	-	18(1)	19(1)	-	19(3)	20(1)	-	17(3)	20(1)
1.4	-	20(2)	20(2)	-	21(2)	21(2)	-	18(3)	18(3)

- Note: Numbers in parenthesis indicates number adult emerged
  DFAE =days to first adult emerged; DHAE =days to highest adult emerged;
  DLAE = days to last adult emerged.

5.2. LC $_{50}$ for 3rd instar larvae

The lethal concentration (mg birbira extract/ml of water) that killed 50% of the DBM eggs, 3rd instars and neonate larvae with the different solvent extracts are described below. Mortality of DBM 3rd instars larvae caused by extracts with WA and AC was very high and similar at all rates and the LC_{50} values of these extracts was 0.08 and 0.19 mg/ml. These values were higher for the extracts of AA and CH which were 0.31 and 0.34 mg/ml, respectively. In contrast, the LC_{50} values were far more for TO and HE extracts which were 0.6 and 0.4 mg/ml, respectively and caused significant larvae killed only at the higher rates applied (Table 12).

Table 12. LC_{50} and 95% confidence limits of the different solvent extracts of birbira seed powder against *P. xylostella* 3rd instar larvae.

Extract type	LC_{50}	95% confidence limits lower & upper limits	Intercept	St. error
Water	0.0865	0.0350, 0.1658	2.1505	0.0872
Acetic acid	0.3114	0.1903, 0.4631	1.0250	0.0587
Acetone	0.1968	0.1078, 0.3107	1.4279	0.0655
Chloroform	0.3466	0.2190, 0.5045	0.9307	0.0552
Toluene	0.6859	0.4711, 0.9925	0.3312	0.0518
Hexane	0.4679	0.3077, 0.6744	0.6672	0.0533

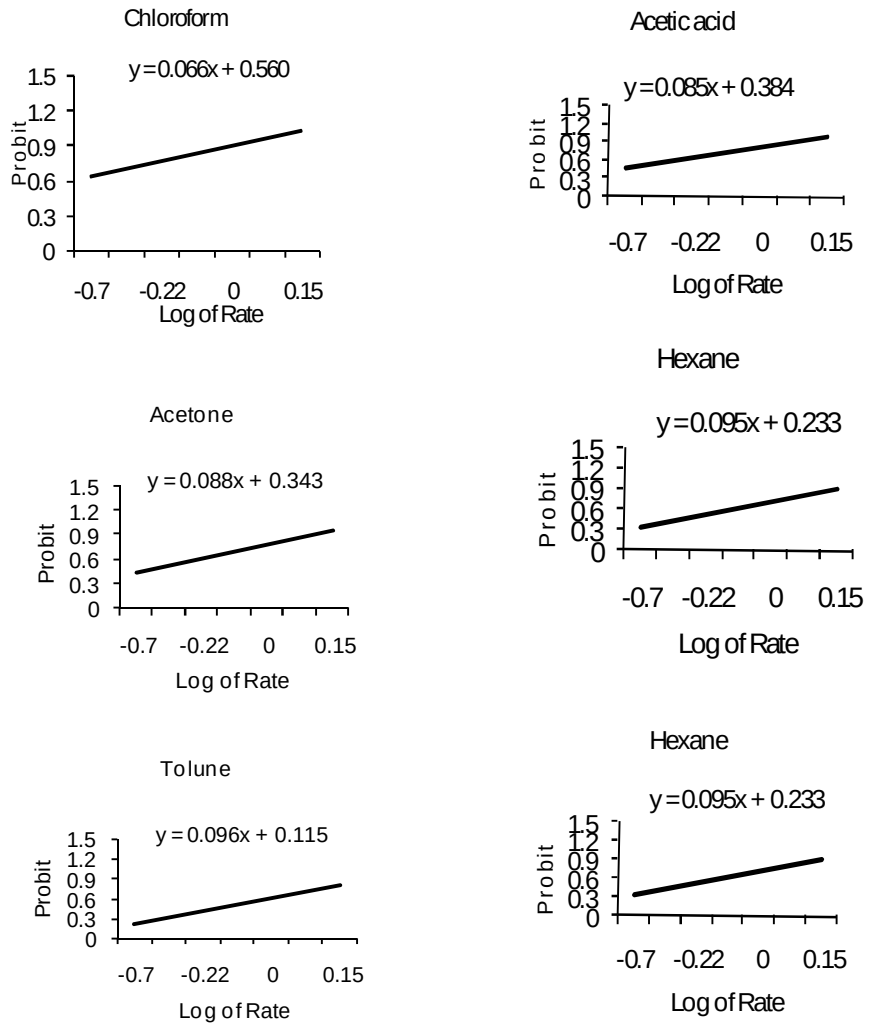

Fig 1. Probit mortality graphs of different solvent extracts of birbira against *P. xylostella* 3rd instars larvae

5.3. Ovicidal effects of birbira extracts

Significant lethal effects of the six solvent extracts of birbira seeds powder on DBM eggs were found 5 days after treatment compared with the control (Annex 11). The numbers of unhatched eggs were increased as the rates of the extracts applied increased in concentration. Eggs unhatchability was higher at 0.4 mg/ml and increased at the rate 1.4 mg/ml for WA, AA, AC and CH extracts. However, significant eggs unhatchability was recorded only at the higher rates for TO and HE extracts.

5.4. LC_{50} of birbira extracts by different solvents against *P. xylosttela* Eggs

The LC_{50} values were much smaller for WA and CH, however this value also smaller for AC and TO as a result mortality of Eggs in these extracts were very high. While the LC_{50} values for the extracts of AA and HE were higher in killing DBM eggs. The values for these extracts were 0.23, 0.31, and 0.50, 0.56 mg/ml, respectively and for AA and HE the values were 1.31 and 1.59 mg/ml, respectively (Table 13).

Table 13. LC_{50} birbira extracts of different solvents against *P. xylosttela* Eggs

Extract type	LC_{50}	95% confidence limits	Intercept	St. error
		lower & upper limits		
Water	0.2366	0.0797, 0.4587	0.0511	0.0506
Acetic acid	1.3163	0.6275, 3.2819	0.0976	0.0495
Acetone	0.5065	0.2358, 0.9666	0.2415	0.0492
Chloroform	0.3128	0.1204 0.5940	0.4128	0.0501
Toluene	0.5640	0.2688, 1.0948	0.2034	0.0495
Hexane	1.5999	0.8358, 4.2857	0.1699	0.0500

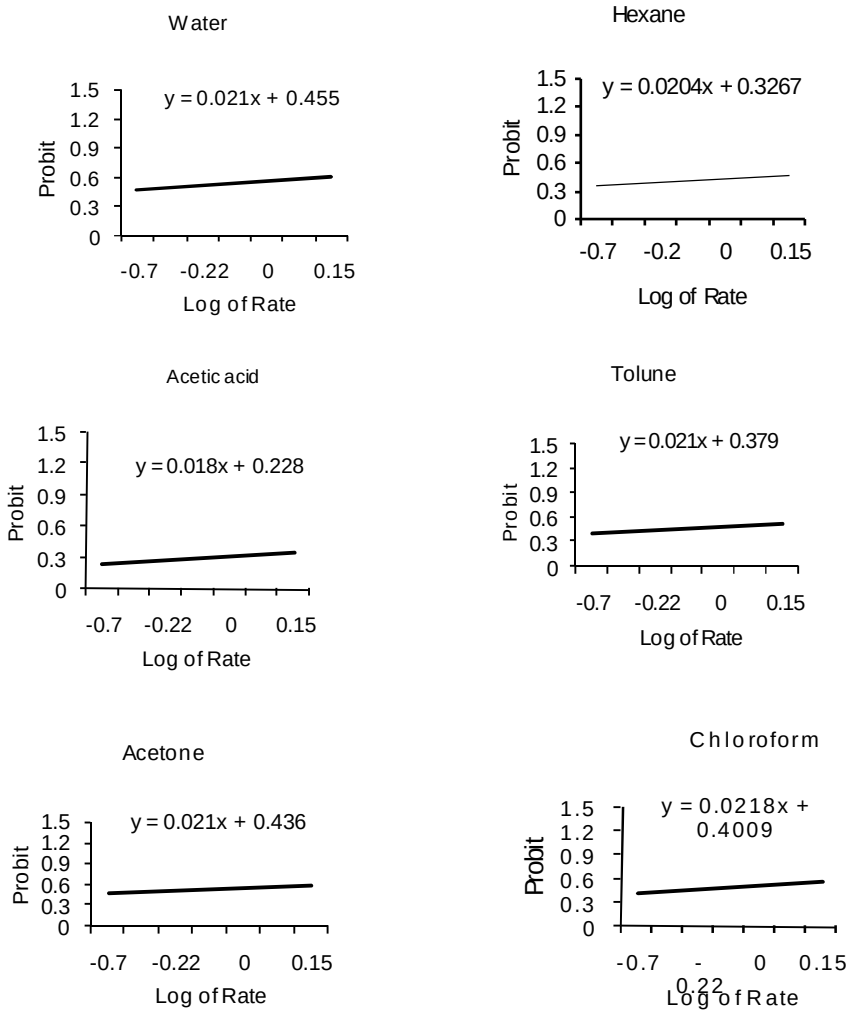

Fig 2. Probit mortality graphs of different solvent extracts of birbira against *P. xylostella* Eggs

5.5. Oviposition deterrence effects

There was a significant difference in the number of eggs laid on leaves treated with the WA extracts at all the rates of application. The within treatment comparison between the rates shows that significantly high number of eggs were laid on leaves that were treated with WA extracted birbira at 0.2 and 0.8 mg/ml ($F_{7,32}$ =6.95,P<0.0001). The highest number of eggs laid for AA extracts at 0.8 mg/ml. This was also the case for AC at the

rate 0.2 and no eggs were laid at 1.4 mg/ml. At 0.2 and 1.2 mg/ml significantly more number of eggs were laid on leaves treated with CH extract and no eggs laid at 1.4 mg/ml ($F_{7, 32}$ =3.31, P<0.009). The highest number of eggs were laid at 0.4 mg/ml for TO and this was significantly different from the other rates ($F_{7, 32}$ =2.69, P<0.026) (Annex 10). There was no significant differences in the number of eggs laid on leaves treated with HE extracts at all rates of applications ($F_{7,32}$ =1.82,P>0.115) (Table 14).

The between treatments comparison at each rate shows that Significantly higher number of eggs were laid on AA extract at the rates (0.4-1.0 mg/ml) treated cabbage leaves and the lowest were laid on WA extract treated leaves (Table 14). Significantly less number of eggs was laid at 1.2 mg/ml on WA treated leaves and the highest was on TO extract. At 1.4 mg/ml significantly less number of eggs was laid on leaves treated with HE extract ($F_{5, 24}$ =1.07, P<0.009) (Annex 21).

Table 14. Mean(±SE) eggs laid by adult, *P. xylostella* on cabbage leaves treated with different solvent extracts of birbira seed powder

Rate	Water	Acetic acid	Acetone	Chloroform	Toluene	Hexane
0	8.00±1.87Aa	8.00±3.11Bd	8.60±1.86Aa	9.00±4.15Cab	7.80±2.31BCa	10.80±1.83Ba
0.2	1.80±0.58Ab	6.20± 3.023Cd	4.80±0.860Ba	6.60±1.435Ca	9.80±4.58Cb	4.80±0.969Bc
0.4	0.40±0.244Bc	14.00±5.029Ac	1.00±0.447Cd	1.60±0.871Cb	12.60±2.85Ab	7.60±1.57Db
0.6	0.20±0.20Ad	14.60±4.308Bc	2.60±2.111Cb	2.00±1.264Cb	2.00±1.04Cc	4.40±1.568Cc
0.8	1.2±0.734Bab	20.20±4.305Ac	0.80±0.374Bbc	2.00±1.140Bb	5.60±2.67Ca	4.60±1.503Cc
1.0	0.0±0.00Aac	11.40±1.326Bc	1.20±0.734Ad	0.00±0.000Ac	4.20±1.35Aa	8.60±4.273Bb
1.2	0.4±0.244Bad	2.00±0.707BCa	0.40±0.400Bab	5.00±1.643Aa	7.40±1.86Aa	5.40±1.805Ac
1.4	0.0±0.00Abc	4.60± 2.293Bb	0.00±0.000Aac	0.00±0.000Ad	2.20±2.66Cb	2.00±1.095Cc

Means followed by the same letter (lowercase) within a column and (uppercase) within a row are not significantly different at P=0.05 (Tukey-Kramer HSD, JMP In 2003).

Eggs laid on inner surface of Petri dishs walls

The numbers of eggs deposited in the inner surface of the Petri dishes were higher at all the rates except for WA, AC and CH extracts at 1.0 and 1.2 mg/ml. No eggs were deposited for these extracts at 1.4mg/ml (Annex 22).

Eggs laid on the inner surface of the cages

The within treatment comparison between the rates shows that significantly high number of eggs were laid on leaves that were treated with WA extracted birbira at 0.6 and 1.4 mg/ml than the other rates and no significant number of eggs were laid at the lower rate and the control. Small numbers of eggs were only laid at 0.2, 0.6 and 1.0 mg/ml for AC. Small numbers of eggs were also only laid on CH extract at 0.2, 0.8 and 1.2 mg/ml

The between treatments comparison at each rate shows higher numbers of eggs deposited in the inner walls of the cages for WA and HE extracts at 0.6 and 1.4 and at 0.4 and 1.4mg/ml respectively, and this was also the case for CH extracted birbira at 0.8 mg/ml. However, significantly very small number of eggs was deposited at these rates (0.6 mg/ml) for AC and no eggs were deposited for CH extracts. In contrast to this, no eggs were deposited in all the rates for AA and TO extracts (Table 15).

Table 15. Number of eggs laid on the inner surface of Cages at different rates and different solvent extracts of birbira.

Rate	WA	AA	AC	CH	TO	HE
0	-	-	-	-	-	-
0.2	-	-	5	6	-	8
0.4	3	-	-	-	-	36
0.6	31	-	2	-	-	-
0.8	-	-	-	23	-	13
1.0	13	-	11	-	-	23
1.2	12	-	-	2	-	-
1.4	34	-	-	-	-	45

Note: WA= water, AA= acetic acid, AC= acetone, CH= chloroform, TO= toluene, HE= hexane

(-) indicates no eggs laid on the inner surface of Cages

5.6. Effects on larvae settlement

The effect of birbira seed powder extracts on the settlements by neonate larvae of *P. xylostella* on treated cabbage leaves is presented in (Table 16) below. The number of neonate larvae settled on leaves that were treated with different solvent extracts of birbira

was more significantly affected by the interaction of extracts. The within treatment comparison between the rates shows that significantly more number of larvae settled at the two lower rates of applications for WA ($F_{7, 32}$ =28.8, P<0.0001) (Annex 9). Significantly fewer larvae settled on cabbage leaves that were treated with AA extracts of birbira, at 0.2 mg/ml within 24 hrs period. No settlement was recorded at all the rates for the AC extract and at 0.8 and 1.2 mg/ml for CH and TO extracts. Higher number of larvae settlement was recorded at 0.2 and 0.4 and 1.0 mg/ml for HE extract.

The between treatments comparison at each rate shows that the highest numbers of larvae were settled at 0.2 and 0.4 mg/ml for HE extract than the other extracts. At 0.4 mg/ml no larvae were settled on leaves treated with for AA and AC extracts. There was no significant difference in the number of larvae settled on leaves treated with TO extract at 0.6 mg/ml ($F_{5, 24}$ =3.53, P>0.015) (Annex 20). At 1.0 and 1.2 mg/ml the differences were not significant whereas at 1.4 mg/ml again significantly more larvae settled on HE extract treated leaves (Table 16).

Table 16. Mean (± SE) settlements recorded on *P. xylostella* larval on cabbage leaves treated with different solvent extracts of birbira seeds kernel powder

Rate	Water	Acetic acid	Acetone	Chloroform	Toluene	Hexane
0	3.00±0.44Aa	3.80±0.37Cb	2.00±0.447Aa	4.00±0.31Cb	4.00±0.44Cc	4.20±0.37Ca
0.2	0.40±0.24Bb	0.20±0.00Ba	0.00±0.00Bb	0.40±0.00Ba	0.40±0.24Bd	2.20±0.86Aa
0.4	0.20±0.20Bc	0.00±0.00Bc	0.00±0.00Bc	0.20±0.00Bd	0.20±0.00Ba	1.40±0.50Aab
0.6	0.00±0.00Ad	0.00±0.00Ad	0.00±0.00Ad	0.40±0.00Ac	0.20±0.00Ab	0.60±0.24Ad
0.8	0.00±0.00Aab	0.00±0.00Aab	0.00±0.00Aab	0.00±0.00Aac	0.00±0.00Abc	0.80±0.37Bc
1.0	0.00±0.00Aac	0.00±0.00Aac	0.00±0.00Aac	0.20±0.00Aab	0.00±0.00Adb	0.20±0.20Abc
1.2	0.00±0.00Aad	0.00±0.00Abc	0.00±0.00Abc	0.00±0.00Adc	0.00±0.00Aac	1.00±0.63Abd
1.4	0.00±0.00Abc	0.00±0.00Abd	0.00±0.00Adb	0.00±0.00Abc	0.00±0.00Aab	0.80±0.37Bb

Means followed by same letter (lowercase) within a column and (uppercase) within a row are not significantly different at P=0.05 (Tukey-Kramer HSD, JMP In 2003).

5.7. LC $_{50}$ of birbira extracts by different solvents against neonate larvae

Mortality of DBM neonate larvae caused by all the solvent extracts was very high. The lethal concentration that killed 50% of the DBM neonate larvae for AA and AC was very smaller and the LC_{50} values were 0.006 and 0.003 mg/ml, respectively. However, these values were higher for WA, CH and TO and these values were 0.01, 0.02 and 0.03 mg/ml, respectively. The LC_{50} values for HE was 0.1 mg/ml which had a much lower effect in killing the DBM neonate larvae (Table 17).

Table 17. LC $_{50}$ and 95% CL of the different solvent extracts from birbira seed powder against *P. xylostella* neonate larvae

Extract type	LC_{50}	95% confidence limits		Intercept	St. error
		Lower & Upper limits			
Water	0.0185	0.0220,	0.0540	2.3571	0.1186
Acetic Acid	0.0061	0.0003,	0.0306	3.0142	0.2028
Acetone	0.0036	0.0000,	0.0333	3.3159	0.3000
Chloroform	0.0288	0.0044,	0.0735	2.0956	0.0984
Toluene	0.0307	0.0047,	0.0775	2.0590	0.0955
Hexane	0.1969	0.0851,	0.3078	0.9625	0.0596

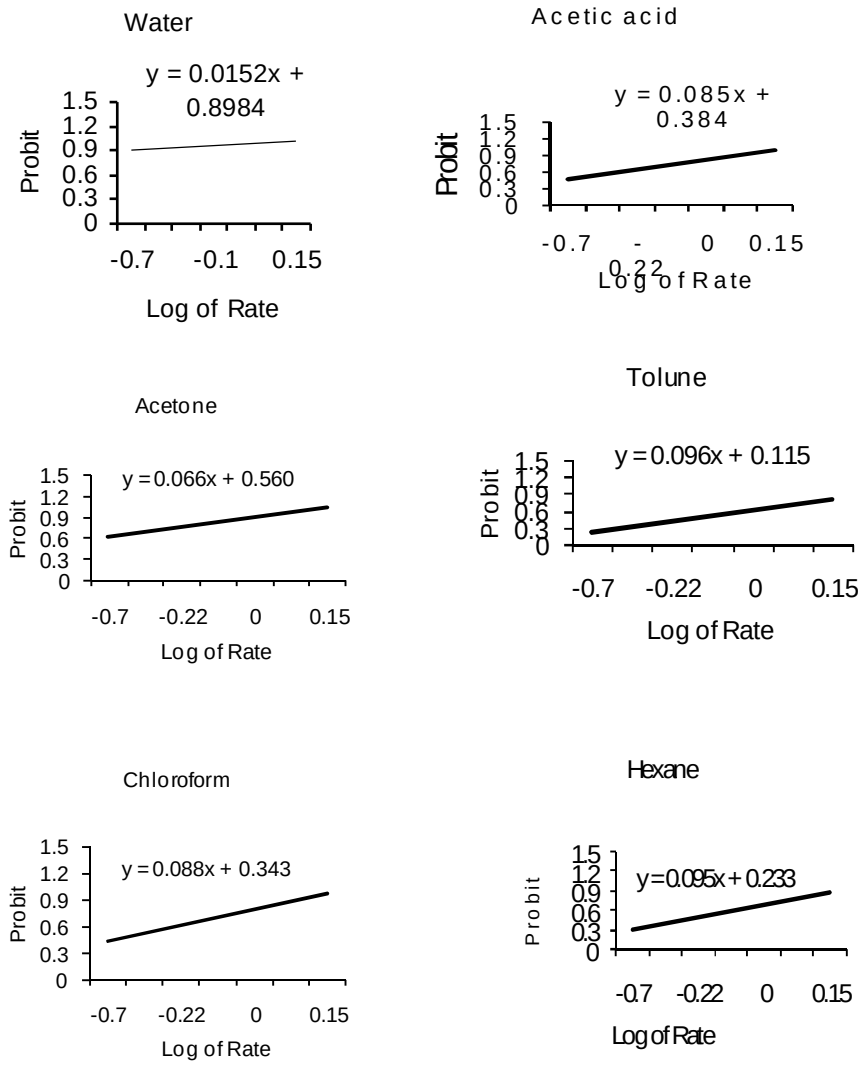

Fig 3. Probit mortality graphs of different solvent extracts of birbira against *P. xylostella* neonate larvae

6. Discussion

The water extracted birbira caused the highest larval mortality at the lower rates. The other extracts which show the highest larval mortality at the higher rates of application include Acetone, Acetic acid and Chloroform. Least mortality was recorded by Toluene and Hexane extracts. This shows that from the different solvents used water was found to be the most effective as compared to all other solvents. Comparison of this result with previous work has shown consistency to (Bekele et al., 2002) where water extracts of birbira caused higher toxicity to all the castes of termites in which 93 to 100% mortality was recorded at all concentration levels.

Days to the 1st larval mortality was observed within a day after the exposure of the 3rd instar larvae on leaves that were treated with different extracts of birbira, except Water. The shortest time to larval mortality were recorded on Water extracted birbira when it was applied at 1.4 mg/ml. Although all solvent extracts have significant insecticidal potency, the potent insecticide concentration in the Water extract might have been higher as compared with the others. Thus this indicates the possibility of producing synergy to better control DBM by contact. The other extracts with shortest day to larvae mortality but brought the effect latter than Water extract were Acetone and Chloroform. Mortality on these extract were significantly higher at the rate 1.0 and 1.2 mg/ml, respectively than the other extracts.

Larval mortality took longer period after fed on treated leaves with all solvent extracts of birbira. Days to last larval mortality after fed on treated leaves with all solvent extracts were observed on the 17th day after treatment application. When 3rd instar larvae of DBM were exposed to the treated leaves with the different extracts of birbira, it was found that the feeding activity was not reduced on the 1st day at the lower doses of the solvent extracts and larvae can survive up to the last day. However, in all solvent extracts, larval mortality significantly increased with increasing rates of application. Comparison of this result with previous work has shown consistency in the study by Chen et al., (1996) the Syringa fruit extracts caused high mortality at all doses tested on. However, at low doses the feeding activities were not reduced on the 1st days when larvae were exposed to the treated leaves. In our study the doses of solvent extracts did not caused significant

mortality but, it did cause a significant reduction in food consumption, which indicates that these extracts have antifeedant properties. At the higher rates of the extracts, larvae became paralyzed, unable to feed and reduced feeding activity and many of the larvae failed to complete development and finally died with increasing mortality

In general, days to highest larval mortality was significantly longer at the lower and medium rates of application and shorter at the highest rate 1.4 mg/ml for all solvent extracts of birbira. The other rates took 8 days to cause same effect. Almost all the larvae that were fed on treated leaves with these extracts died within a few days of application. Larval development time was also significantly prolonged by solvent extracts of birbira when larvae were only exposed to the treatments at the 3rd instar. The assumptions for this may be that when 3rd instar larvae exposed to the solvent extracts of birbira appear to feed much less than expected thus their development was slowed as a result many died before they could pupate. Hence the days to highest larval mortality took significantly longer period of time probably due to the slow acting effect of the potent insecticides in birbira on feeding DBM larvae.

When DBM 3rd instar larvae were exposed to leaves treated with solvent extracts, development was prolonged. Larvae took longer period to reach the 4th instar and the pupal stage. Results of this study showed that 3rd instar larvae that were developed into pupa in the control treated leaves took only 8 days to pupate. However, no larvae were pupated at this day at all rates of application of the extracts. The period of pupation had significantly increased as the rate of extracts applied increased for all the treatments except for WA, AA and AC at the highest rates. Results from this study confirm that in addition to WA, AA and AC the organic extracts such as TO and HE from birbira seeds also have a sever impact on the ability of *P. xylostella* to moult which resulted in significant larval mortality, reduced and delayed pupation. The resulted pupal size was found to be significantly lower on the treated cabbage leaves than on the control, which could be explained by reduced feeding. The lower rate of development and failure to moult has been previously reported for *P. xylostella* treated with Neem extracts (Isman, 1999; Liang et al., 2003).

Water and Acetic acid allowed fewer larvae to pupate at the rates between 0.8 and 1.4 mg/ml. However, AC prevented pupation at the rate between 0.8 and 1.4 mg/ml. Comparison of the present results with previous work has shown consistency result to that by Bekele Jembere (2002) who reported that the AC extract was the most toxic to Maize weevil. This result indicated that AC is efficient in extracting the active compound against the weevil. CH and HE also allowed small number of larvae to pupate. However, the total number of larvae pupated at the day generally decreased as the rate increases for these extracts. In contrast, the results of the present study showed that the total number of larvae pupated were significantly higher in TO extract at all rates of application (Table 9). The assumption for this may be that the active compound is less in amount in this extract.

The number of adults that were emerged from pupae on the untreated leaves and leaves that were treated with different extracts at the lower rates were higher in number. Most of the adults that were emerged from pupae on the control were on the 14^{th} days. However, DBM appeared to be affected by solvent extracts of birbira in the larval stages of development. Pupal mortality was high with many adults failed to emerge. On leaves that were treated with all solvent extracts of birbira adult emergence occurred after 17 days, but the numbers were very low, some of the adults that did emerge from pupae that had been exposed to the solvent extracts of birbira were very small in size, deformed with shorter wings or wings that failed to expand properly. Comparison of the result with previous work has shown consistency result to Charlston (2004) who reported the *Melia azedarach* and *Azadirachta* indica were both appears to have antifeedent and growth inhibition properties against *P. xylostella.*

Polar solvent extracts of birbira appear to significantly deter oviposition by DBM adult. It can also be noted that the deterrence shown by the polar solvent extracts was considerably more than that of the non polar extracts of similar concentration. Result showed that the non polar solvent (TO and HE) extracts of birbira exhibited significantly lower oviposition deterrence on DBM as indicated by higher number of eggs oviposited

on treated cabbage leaves. The possible explanation for this higher number of eggs oviposited on treated cabbage leaves may be the odor or volatile of birbira extracted by the non polar solvents might be weaker than that of the polar solvents. In the present study it was found that significantly fewer DBM eggs were oviposited on WA, AC and CH extracts. Full deterrence was recorded at 1.0 and 1.4 mg/ml for these extracts, suggesting that it might contain most of the volatile constituents in birbira.

The reduced oviposition on treated leaves may be related to the deterrent effect of the extracts to the gravid adults. In these extracts, it was found that oviposition deterrence increased with an increase in the rate of application of the extract. Comparison of the current result with previous work has shown consistency. For instances the result of *Acorus calamus* L. from Nair and Thomas (2001) to Melon fly (*Bacterocera cucurbitae* Coq.) revealed that *Acorus calamus* L. increase deterrence with increasing concentration of the extracts.

Crude aqueous and organic extracts from Neem seed kernel were also reported to deter oviposition by melon and oriental fruit flies, but pure azadirachtin failed to deter egg laying (Singh and Singh 1998). This was also confirmed by Liang *et al*., (2003) that Neem based commercial insecticides did not deter oviposition by DBM. These show the importance of crude extracts as deterrents and hence magnify their potential application in the crude form for pest control.

Number of eggs deposited on the inner surface of the Petri dishes were higher for all the rates of extracts and no eggs were deposited for WA, AC and CH extracts at the higher rates, suggested that there may have been sufficiently large amount of the active compounds released over 72 hrs and deterred the gravid DBM females from laying their eggs even on the inner walls of the Petri dishes.

The number of eggs deposited in the inner walls of the cages was higher for WA, CH and HE and significantly very small number of eggs for AC extract. In contrast to this no eggs were deposited in all the rates for AA and TO extracts. What is very interesting to notice is that the deposition of eggs on the inside surface of the Petri dishes and cages decreased significantly like on the leaves with the increasing rates of application of the

extract and no eggs were deposited away from the leaves at 1.4 mg/ml. This shows that the higher rates of crude extract with polar solvents completely arrested the oviposition response by DBM adults.

Significantly fewer larvae settled on cabbage leaves that were treated with WA extracts of birbira, at the lower two rates, i.e., 0.2 and 0.4 mg/ml and at 0.2 mg/ml for AA extract. The other extract which inhibits full settlement recorded at all the rates is AC extracted birbira within observation period of 24 hrs. However in contrast to this, higher number of larvae settlement was recorded at 0.2 and 0.4 mg/ml for HE extracted birbira. Previous study had shown consistency from Koschier and Sedy (2003) that Rosemary oil application at 1% concentration inhibit settlements by larvae of *Thrips tabaci* over a 4 hrs period.

Significant lethal effects of the solvent extracts of birbira on *P. xylostella* eggs were found five days after treatment compared with the water treated controls. Egg hatching was significantly lower at the tested concentration. This was found to be because of the ovicidal action of the solvent extraction of birbira. Microscopic examinations of the unhatched eggs revealed that the majority of the eggs were killed at the very early stages of development. It was also found that some neonates died before they hatched from the eggs, implying that the insecticides on the egg surface were toxic to the neonates when they contacted these materials while hatching.

For the DBM 3rd instar and neonate larvae and eggs treated with the six extracts of Birbira, mortality was confirmed to be significantly dependent on the rates of application of each extract. Therefore, mortality found after the correction for the natural death of the DBM larvae and eggs in all the extracts is attributable to the concentration gradient dependent biocidal effect of the extracts. But, the lethal concentration that killed 50% of the DBM larvae and eggs was different for all the treatments. Mortality of 3rd instar larvae caused by extracts with WA and AC was very high and similar at all the rates. The LC_{50} values for these extract were much smaller. These values were also smaller for AA and CH extracts. In contrast, these values were much higher for the extracts by TO and HE than the other extracts, which had a much lower killing effect.

Mortality of the DBM neonate larvae caused by all solvent extracts were very high except HE extract and their LC_{50} values were smaller but have a much higher killing effect. Mortality of the DBM eggs caused by extracts with WA, AC and CH was very high and similar at all the rates. The LC_{50} values for these extract were much smaller. TO extracted birbira had higher LC_{50} values and had lower killing effect. In contrast, these values were much higher for the extracts by AA and HE than the other extracts, which had a much lower killing effect.

7. Conclusions and Recommendations

7.1. Conclusions

- Results of this study suggested that solvent extracts from Birbira, with solvents of different polarity have shown strong effect on the biological activities of the test insect (DBM).

- Results of this study suggested that solvent extracts from Birbira, with solvents of different polarity had ovicidal and larvicidal effects on DBM.

- Polar solvent extracts of Birbira were toxic and have antifidant effect on the larvae of DBM, while the non polar solvent extracts were less toxic and less deterrent the feeding and oviposition activities of the test insect.

- Polar solvent extracts of Birbira were toxic to DBM eggs and neonate larvae and caused high mortality at all rates of applications for all the solvent extracts.

- Birbira seeds kernel contains natural insecticides that can be extracted using solvents with different polarity and act on insects by contact, as feeding and oviposition deterrence.

7.2. Recommendations

- In this study solvent extracts of Birbira with different polarities were observed causing feeding and oviposition deterrence, eggs and neonate larval mortality effects on the test insects. However, to enhance the toxic effect of the plant, further investigations are required particularly on the chemical composition of the plant and methods of formulation of the active plant material.

- Plant derived compounds are easily degradable and their effect in the environment is least compaired to conventional pesticides. Thus, development of bio-pesticides using these products should be encouraged to ensure the safety of our environment and our selves.

- The gap between results from the laboratory studies and what actually happens in the field can be large. Therefore, field experiment is essential to validate this laboratory finding.

- The toxicity effect of solvent extracts of Birbira with different polarity is significant on the eggs and the neonate. Therefore, it is important to use these botanicals at the earliest stages of development as well.

- Further investigation of the impact of this botanical plant on the behavior of DBM, biology and behavior of its natural enemies are important aspects to be studied.

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9. Appendices

Annex 1. Analysis of Variance (ANOVA) for corrected mortality by rate

Extract Type	Source	DF	Sum of Squares	Mean square	F-Ratio	P>F
Water	Within	6	476.866	79.477	0.8309	0.556
	Among	27	2582.677	95.6547		
	Total	33	3059.544			
Acetic acid	Within	6	19416.502	3236.08	13.238	<0.0001
	Among	27	6600.176	244.45		
	Total	33	26016.678			
Acetone	Within	6	11035.460	1839.24	23.073	<0.0001
	Among	27	2152.231	79.71		
	Total	33	13187.692			
Chloroform	Within	6	11341.607	1890.27	4.536	0.0028
	Among	27	10832.898	416.65		
	Total	33	22174.504			
Tolune	Within	6	33513.617	5585.60	7.630	<0.0001
	Among	27	19764.657	732.02		
	Total	33	53278.275			
Hexane	Within	6	12831.398	2138.57	2.5761	0.0400
	Among	27	23244.098	830.15		
	Total	33	36075.496			

Annex 2. Analysis of Variance (ANOVA) for DH/TM by rate

Extract Type	Source	DF	Sum of Squares	Mean square	F-Ratio	P>F
Water	Within	7	210.3750	30.0536	4.1525	<0.0024
	Among	32	231.6000	7.2375		
	Total	39	441.9750			
Acetic acid	Within	7	108.1750	15.4536	1.719	0.139
	Among	32	287.6000	8.9875		
	Total	39	395.7750			
Acetone	Within	7	312.5750	44.6536	9.5772	<0.0001
	Among	32	149.2000	4.6625		
	Total	39	461.7750			
Chloroform	Within	7	232.0000	33.1429	3.2334	<0.0104
	Among	32	328.0000	10.2500		
	Total	39	560.0000			
Tolune	Within	7	372.8000	53.2571	4.8860	<0.0008
	Among	32	348.8000	10.9000		
	Total	39	721.6000			
Hexane	Within	7	125.6000	17.9429	1.8862	0.1048
	Among	32	304.4000	9.5125		
	Total	39	430.0000			

Annex 3. Analysis of Variance (ANOVA) for days to 1st larval mortality by rate

Extract Type	Source	DF	Sum of Squares	Mean square	F-Ratio	P>F
Water	Within	7	2.1750	0.3107	1.7755	0.1268
	Among	32	5.6000	0.1750		
	Total	39	7.7750			
Acetic acid	Within	7	2.9750	0.4250	0.8095	0.5859
	Among	32	16.800	0.5250		
	Total	39	19.775			
Acetone	Within	7	9.2000	1.3742	2.5034	<0.0360
	Among	32	16.800	0.5250		
	Total	39	26.000			
Chloroform	Within	7	3.5750	0.5107	1.9456	0.0945
	Among	32	8.4000	0.2625		
	Total	39	11.975			
Tolune	Within	7	2.7000	0.3857	0.7347	0.6442
	Among	32	16.800	0.5250		
	Total	39	19.500			
Hexane	Within	7	4.7750	0.6821	3.0317	<0.0146
	Among	32	7.2000	0.2250		
	Total	39	11.975			

Annex 4. Analysis of Variance (ANOVA) for days to last larval mortality by rate

Extract Type	Source	DF	Sum of Squares	Mean square	F-Ratio	P>F
Water	Within	7	63.9750	9.1392	33.2338	<0.0001
	Among	32	8.8000	0.2750		
	Total	39	72.7750			
Acetic acid	Within	7	54.7750	7.8250	10.7931	<0.0001
	Among	32	23.2000	0.7250		
	Total	39	77.9750			
Acetone	Within	7	49.1750	7.0250	11.7083	<0.0001
	Among	32	19.2000	0.6000		
	Total	39	68.3750			
Chloroform	Within	7	52.5750	7.5107	10.5414	<0.0001
	Among	32	22.8000	0.7125		
	Total	39	75.3750			
Tolune	Within	7	59.5000	8.500	6.5385	<0.0001
	Among	32	41.6000	1.300		
	Total	39	101.100			
Hexane	Within	7	32.9750	4.7107	4.3821	<0.0017
	Among	32	34.4000	1.0750		
	Total	39	67.3750			

Annex 5. Analysis of Variance (ANOVA) for number of pupae 1st date by rate

Extract Type	Source	DF	Sum of Squares	Mean square	F-Ratio	P>F
Water	Within	7	17.5000	2.5000	13.333	<0.0001
	Among	32	6.0000	0.1875		
	Total	39	23.5000			
Acetic acid	Within	7	10.3750	1.4821	13.1746	<0.0001
	Among	32	3.6000	0.1125		
	Total	39	13.9750			
Acetone	Within	7	17.5000	2.5000	40.000	<0.0001
	Among	32	2.0000	0.0625		
	Total	39	19.5000			
Chloroform	Within	7	4.3750	0.6250	3.3333	<0.008
	Among	32	6.0000	0.1875		
	Total	39	10.3750			
Tolune	Within	7	35.5000	5.0714	4.2262	<0.0021
	Among	32	38.4000	1.2000		
	Total	39	73.9000			
Hexane	Within	7	4.30000	0.6142	2.8900	<0.018
	Among	32	6.50000	0.2125		
	Total	39	11.1000			

Annex 6. Analysis of Variance (ANOVA) for highest pupae by rate

Extract Type	Source	DF	Sum of Squares	Mean square	F-Ratio	P>F
Water	Within	7	26.0000	3.7142	19.809	<0.0001
	Among	32	6.0000	0.1875		
	Total	39	32.0000			
Acetic acid	Within	7	15.1000	2.1571	8.2177	<0.0001
	Among	32	8.4000	0.2625		
	Total	39	23.5000			
Acetone	Within	7	28.3750	4.0535	29.4805	<0.0001
	Among	32	4.4000	0.1375		
	Total	39	32.7750			
Chloroform	Within	7	25.1750	3.5964	12.509	<0.0001
	Among	32	9.2000	0.2875		
	Total	39	34.3750			
Tolune	Within	7	30.8000	4.4000	5.1014	<0.0006
	Among	32	27.6000	0.8625		
	Total	39	58.4000			
Hexane	Within	7	18.5750	2.6535	5.1777	<0.0005
	Among	32	16.4000	0.5125		
	Total	39	34.9750			

Annex 7. Analysis of Variance (ANOVA) for total pupae by rate

Extract Type	Source	DF	Sum of Squares	Mean square	F-Ratio	P>F
Water	Within	7	63.6000	9.0857	33.0390	<0.0001
	Among	32	8.8000	0.2750		
	Total	39	72.4000			
Acetic acid	Within	7	54.9750	7.8535	8.9755	<0.0001
	Among	32	28.0000	0.8750		
	Total	39	82.9750			
Acetone	Within	7	49.7750	7.1107	20.3163	<0.0001
	Among	32	11.2000	0.3500		
	Total	39	60.9750			
Chloroform	Within	7	53.9000	7.7000	10.2667	<0.0001
	Among	32	24.0000	0.7500		
	Total	39	77.9000			
Tolune	Within	7	62.3750	8.9107	7.0580	<0.0001
	Among	32	40.4000	1.2625		
	Total	39	102.775			
Hexane	Within	7	35.1000	5.0142	4.3602	<0.0017
	Among	32	36.8000	1.1500		
	Total	39	71.9000			

Annex 8. Analysis of Variance (ANOVA) for percent emerged by rate

Extract Type	Source	DF	Sum of Squares	Mean square	F-Ratio	P>F
Water	Within	7	28150.00	4021.43	33.5119	<0.0001
	Among	32	3840.00	120.00		
	Total	39	31990.00			
Acetic acid	Within	7	23990.00	3427.14	21.4196	<0.0001
	Among	32	5120.00	160.00		
	Total	39	29110.00			
Acetone	Within	7	22550.00	3221.43	30.6803	<0.0001
	Among	32	3360.00	105.00		
	Total	39	25910.00			
Chloroform	Within	7	24600.00	3514.29	10.9821	<0.0001
	Among	32	10240.00	320.00		
	Total	39	34840.00			
Tolune	Within	7	25750.00	3678.57	7.2843	<0.0001
	Among	32	16160.00	505.00		
	Total	39	41910.00			
Hexane	Within	7	15510.00	2215.71	4.1032	<0.0026
	Among	32	17280.00	540.00		
	Total	39	32790.00			

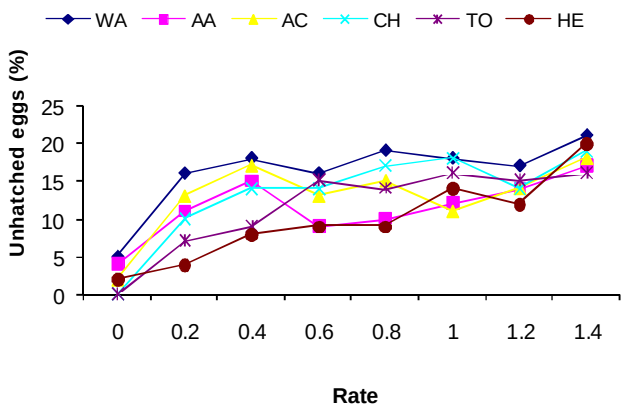
Annex 9. Analysis of Variance (ANOVA) for number larvae settled by rate

Extract Type	Source	DF	Sum of Squares	Mean square	F-Ratio	P>F
Water	Within	7	37.9000	5.4142	28.8762	<0.0001
	Among	32	6.0000	0.1875		
	Total	39	43.9000			
Acetic acid	Within	7	62.4000	8.9142	79.238	<0.0001
	Among	32	3.6000	0.1125		
	Total	39	66.0000			
Acetone	Within	7	17.5000	2.5000	20.000	<0.0001
	Among	32	4.0000	0.1250		
	Total	39	21.5000			
Chloroform	Within	7	65.1000	9.3000	49.6000	<0.0001
	Among	32	6.0000	0.1875		
	Total	39	71.1000			
Tolune	Within	7	68.7000	9.8142	60.3956	<0.0001
	Among	32	5.2000	0.1625		
	Total	39	73.9000			
Hexane	Within	7	57.2000	8.1714	6.8095	<0.0001
	Among	32	38.4000	1.2000		
	Total	39	95.0000			

Annex 10. Analysis of Variance (ANOVA) for eggs laid on leaves by rate

Extract Type	Source	DF	Sum of Squares	Mean square	F-Ratio	P>F
Water	Within	7	243.9750	34.8536	6.9533	<0.0001
	Among	32	160.4000	5.0125		
	Total	39	404.3750			
Acetic acid	Within	7	1273.175	181.882	3.2786	<0.0097
	Among	32	1775.200	55.475		
	Total	39	3048.375			
Acetone	Within	7	299.7750	42.8250	7.0639	<0.0001
	Among	32	194.0000	6.0625		
	Total	39	493.7750			
Chloroform	Within	7	371.5750	53.0821	3.3150	<0.0091
	Among	32	512.4000	16.0125		
	Total	39	883.9750			
Tolune	Within	7	649.9000	92.8429	2.6911	<0.0260
	Among	32	1104.000	34.5000		
	Total	39	1753.900			
Hexane	Within	7	273.3750	39.0536	1.8281	0.1158
	Among	32	683.6000	21.3625		
	Total	39	956.9750			

Annex 11. Ovicidal effects of birbira extracts.



Number of eggs unhatchability of P. xylostella on cabbage leaves treated with different solvent extracts of birbira

Annex 12. Analysis of Variance (ANOVA) for corrected mortality by treatment

Rates	Source	DF	Sum of Squares	Mean square	F-Ratio	P>F
0	Within	5	27425.23	1959.77	3.18	0.0001
	Among	24	12042.56	614.35		
	Total	29	39467.79			
0.2	Within	5	25659.38	5131.88	8.759	0.0001
	Among	24	14061.24	585.89		
	Total	29	39720.62			
0.4	Within	5	16548.27	3309.65	5.323	0.0020
	Among	24	14922.13	621.76		
	Total	29	31470.40			
0.6	Within	5	26703.748	5340.75	14.328	0.0001
	Among	23	8573.055	372.74		
	Total	28	32276.803			
0.8	Within	5	4688.001	937.6000	2.302	0.0832
	Among	20	8142.608	407.130		
	Total	25	12830.610			
1.0	Within	5	2510.9365	502.187	1.680	0.177
	Among	24	7174.1043	298.921		
	Total	29	9685.0409			
1.2	Within	5	6665.732	1333.15	3.5766	0.015
	Among	23	8573.055	372.74		
	Total	28	15238.785			
1.4	Within	5	2702.246	540.449	3.4769	0.0167
	Among	24	3730.5343	155.439		
	Total	29	6432.7802			

Annex 13. Analysis of Variance (ANOVA) for DH/TM by treatment

Rates	Source	DF	Sum of Squares	Mean square	F-Ratio	P>F
0	Within	5	61.8667	12.3733	1.0999	0.3863
	Among	24	270.000	11.2500		
	Total	29	331.8667			
0.2	Within	5	139.4667	27.8933	4.6489	0.004
	Among	24	144.000	6.0000		
	Total	29	283.46667			
0.4	Within	5	274.16667	54.8333	6.8116	0.0004
	Among	24	193.2000	8.0500		
	Total	29	467.36667			
0.6	Within	5	89.1000	17.8200	7.8498	0.1411
	Among	23	231.2000	9.6333		
	Total	28	320.3000			
0.8	Within	5	61.5000	12.3000	1.0885	0.3920
	Among	20	271.2000	11.3000		
	Total	25	332.7000			
1.0	Within	5	59.36667	11.8733	2.1139	0.0985
	Among	24	134.8000	5.6167		
	Total	29	194.1666			
1.2	Within	5	100.3000	20.060	2.5609	0.0541
	Among	23	188.0000	7.8333		
	Total	28	288.3000			
1.4	Within	5	104.000	20.8000	2.2983	0.0768
	Among	24	217.200	9.05000		
	Total	29	321.2000			

Annex 14. Analysis of Variance (ANOVA) for the first larval mortality by treatment

Rates	Source	DF	Sum of Squares	Mean square	F-Ratio	P>F
0.4	Within	5	1.500	0.30	6.00	0.0001
	Among	24	1.200	0.05		
	Total	29	2.700			
0.6	Within	5	4.1666	0.833	10.00	0.0001
	Among	24	2.0000	0.0833		
	Total	29	6.1666			
0.8	Within	5	0.1667	0.033	1.00	0.4389
	Among	24	0.8000	0.033		
	Total	29	0.9666			
1.0	Within	5	0.666	0.13	2.66	0.0471
	Among	24	1.200	0.05		
	Total	29	1.860			
1.2	Within	5	0.0666	0.213	1.1636	0.3556
	Among	24	4.4000	0.183		
	Total	29	5.4667			
1.4	Within	5	1.5000	0.300	2.2500	0.082
	Among	24	3.2000	0.133		
	Total	29	4.7000			

Annex 15. Analysis of Variance (ANOVA) for last larval mortality by treatment

Rates	Source	DF	Sum of Squares	Mean square	F-Ratio	P>F
0	Within	5	5.8666	1.173	1.760	0.1594
	Among	24	16.000	0.666		
	Total	29	21.866			
0.2	Within	5	35.7667	7.153	8.759	0.0001
	Among	24	19.6000	0.8166		
	Total	29	55.3667			
0.4	Within	5	23.06667	4.6133	5.323	0.002
	Among	24	20.8000	0.8667		
	Total	29	43.8666			
0.6	Within	5	28.000	5.600	5.7931	0.0012
	Among	23	23.200	0.9667		
	Total	28	51.200			
0.8	Within	5	4.2666	0.8533	0.6481	0.6656
	Among	20	31.600	1.3166		
	Total	25	35.866			
1.0	Within	5	3.5000	0.7000	1.680	0.1778
	Among	24	10.000	0.4166		
	Total	29	13.500			
1.2	Within	5	8.9667	1.7933	2.9889	0.0309
	Among	23	14.400	0.6000		
	Total	28	23.366			
1.4	Within	5	3.7666	0.7533	3.4769	0.0167
	Among	24	5.2000	0.2166		
	Total	29	8.9667			

Annex 16. Analysis of Variance (ANOVA) number of pupae 1st date by treatment

Rates	Source	DF	Sum of Squares	Mean square	F-Ratio	P>F
0	Within	5	14.1666	2.830	2.931	0.0333
	Among	24	23.2000	0.966		
	Total	29	37.3666			
0.2	Within	5	5.7660	1.153	2.035	0.109
	Among	24	13.6000	0.566		
	Total	29	19.3666			
0.4	Within	5	13.066	2.613	3.563	0.015
	Among	24	17.600	0.733		
	Total	29	30.666			
0.6	Within	5	5.766	1.1533	7.688	0.0002
	Among	23	3.600	0.1500		
	Total	28	9.366			
0.8	Within	5	0.1666	0.033	1.00	0.4389
	Among	20	0.8000	0.033		
	Total	25	0.9666			
1.0	Within	5	0.666	0.1333	1.000	0.4389
	Among	24	3.200	0.1333		
	Total	29	3.866			
1.2	Within	5	2.660	0.533	16.00	0.0001
	Among	23	0.800	0.033		
	Total	28	3.466			

Annex 17. Analysis of Variance (ANOVA) for highest pupae by treatment

Rates	Source	DF	Sum of Squares	Mean square	F-Ratio	P>F
0	Within	5	5.3666	1.073	4.293	0.006
	Among	24	6.0000	0.250		
	Total	29	11.366			
0.2	Within	5	14.66	2.93	6.518	0.0006
	Among	24	10.80	0.45		
	Total	29	25.46			
0.4	Within	5	15.366	3.073	4.983	0.0029
	Among	24	14.800	0.616		
	Total	29	30.166			
0.6	Within	5	9.20	1.840	5.018	0.0028
	Among	23	8.80	0.366		
	Total	28	18.00			
0.8	Within	5	5.60	1.120	2.80	0.0395
	Among	20	9.60	0.40		
	Total	25	15.20			
1.0	Within	5	2.966	0.593	1.369	0.270
	Among	24	10.40	0.433		
	Total	29	13.366			
1.2	Within	5	3.60	0.720	2.273	0.0794
	Among	23	7.60	0.316		
	Total	28	11.20			
1.4	Within	5	2.66	0.533	3.200	0.0238
	Among	24	4.00	0.166		
	Total	29	6.66			

Annex 18. Analysis of Variance (ANOVA) for total pupae by treatment

Rates	Source	DF	Sum of Squares	Mean square	F-Ratio	P>F
0	Within	5	3.866	0.773	1.031	0.421
	Among	24	18.000	0.750		
	Total	29	21.866			
0.2	Within	5	35.500	7.100	6.871	0.0004
	Among	24	24.800	1.033		
	Total	29	60.300			
0.4	Within	5	23.366	4.673	5.392	0.0018
	Among	24	20.800	0.866		
	Total	29	44.166			
0.6	Within	5	25.366	5.073	5.5345	0.0016
	Among	23	22.000	0.9166		
	Total	28	47.366			
0.8	Within	5	3.366	0.673	0.511	0.764
	Among	20	31.600	1.316		
	Total	25	34.966			
1.0	Within	5	2.966	0.5933	1.3692	0.270
	Among	24	10.400	0.4333		
	Total	29	13.366			
1.2	Within	5	9.766	1.9533	3.005	0.0303
	Among	23	15.600	0.650		
	Total	28	25.366			
1.4	Within	5	2.966	0.593	2.373	0.0695
	Among	24	6.000	0.250		
	Total	29	8.966			

Annex 19. Analysis of Variance (ANOVA) for percent emerged by treatment

Rates	Source	DF	Sum of Squares	Mean square	F-Ratio	P>F
0	Within	5	1386.666	277.333	1.1886	0.3441
	Among	24	5600.000	233.333		
	Total	29	6986.666			
0.2	Within	5	15626.667	3125.33	4.833	0.0034
	Among	24	15520.000	646.67		
	Total	29	31146.667			
0.4	Within	5	11106.667	2221.33	10.097	0.0001
	Among	24	5280.000	220.00		
	Total	29	16386.667			
0.6	Within	5	8746.667	1749.33	4.3733	0.0057
	Among	23	9600.000	400.00		
	Total	28	18346.667			
0.8	Within	5	1506.6667	301.33		
	Among	20	7360.0000	306.667	0.9826	0.4486
	Total	25	8866.6667			
1.0	Within	5	1280.00	256.000	1.5360	0.2162
	Among	24	4000.00	166.667		
	Total	29	5280.00			
1.2	Within	5	3906.667	781.333	3.0051	0.0303
	Among	23	6240.000	260.000		
	Total	28	10146.667			
1.4	Within	5	1066.6667	213.333	2.1333	0.0960
	Among	24	2400.0000	100.000		
	Total	29	3466.6667			

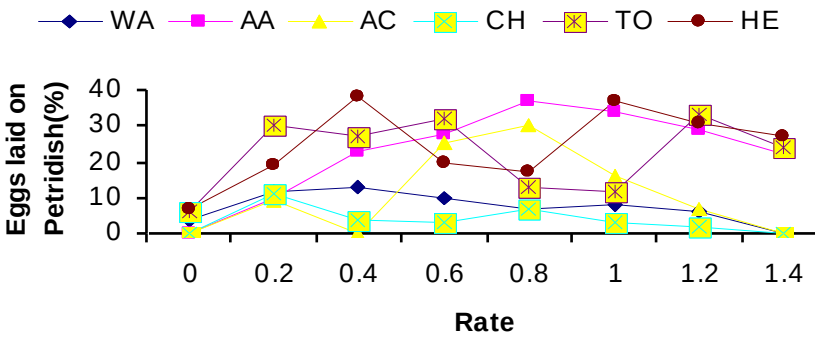
Annex 20. Analysis of Variance (ANOVA) for larvae settled by treatment

Rates	Source	DF	Sum of Squares	Mean square	F-Ratio	P>F
0	Within	5	17.900	3.500	4.383	0.0056
	Among	24	19.600	0.816		
	Total	29	37.500			
0.2	Within	5	16.000	3.200	4.00	0.008
	Among	24	19.200	0.800		
	Total	29	35.200			
0.4	Within	5	7.500	10.50	5.294	0.002
	Among	24	6.800	0.283		
	Total	29	14.30			
0.6	Within	5	1.766	0.350	3.533	0.015
	Among	23	2.400	0.100		
	Total	28	4.166			
0.8	Within	5	2.66	0.533	4.571	0.004
	Among	20	2.80	0.116		
	Total	25	5.46			
1.0	Within	5	0.266	0.053	0.80	0.560
	Among	24	1.600	0.060		
	Total	29	1.866			
1.2	Within	5	4.16	0.833	2.500	0.0587
	Among	23	8.00	0.333		
	Total	28	12.16			
1.4	Within	5	2.66	0.533	4.571	0.0045
	Among	24	2.80	0.116		
	Total	29	5.46			

Annex 21. Analysis of Variance (ANOVA) for Eggs laid on leaves by treatment

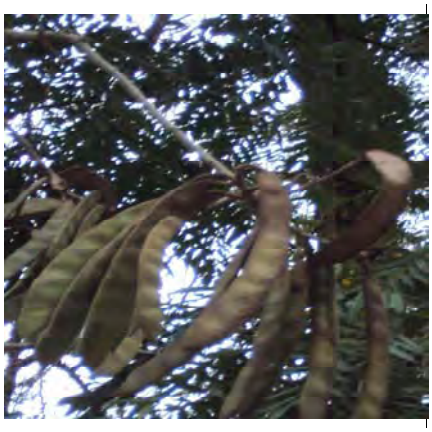
Rates	Source	DF	Sum of Squares	Mean square	F-Ratio	P>F
0	Within	5	276.42	29.53	2.30	0.0001
	Among	24	192.80	12.84		
	Total	29	469.22			
0.2	Within	5	1896.24	563.615	3.77	0.0001
	Among	24	2235.70	149.500		
	Total	29	4132.94			
0.4	Within	5	392.05	430.61	5.09	0.001
	Among	24	284.76	84.60		
	Total	29	676.81			
0.6	Within	5	481.602	1052.43	2.80	0.008
	Among	23	627.400	375.87		
	Total	28	1109.00			
0.8	Within	5	729.27	1303.63	1.82	0.076
	Among	20	2314.00	716.28		
	Total	25	3043.27			
1.0	Within	5	343.15	586.058	0.69	0.207
	Among	24	436.50	849.36		
	Total	29	779.65			
1.2	Within	5	253.12	31.954	3.44	0.0001
	Among	23	126.10	9.27		
	Total	28	379.22			
1.4	Within	5	251.20	27.359	1.073	0.009
	Among	24	109.40	25.498		
	Total	29	360.60			

Annex 22. Eggs laid on inner surface of Petri dish walls



Number of eggs laid by *P. xylostella* adults on inner surface of Petri dish walls.

Annex 23. The test plant, Birbira, *M. ferruginea* (Hochst) Baker



Matured Birbira Pods

Birbira seed coats

Birbira seed cotyledons

Mortar and Pestle

Annex 24. Cabbage, *Brassica oleracea* L. *var Capitata*

Small sized plastic pots

Cabbage seedlings

Bigger cage for rearing the test insect

Annex 25. Feeding and oviposition bioassays

Feeding deterrence bioassay

oviposition deterrence bioassay