ASPECTS OF BIO-INTENSIVE PEA APHID, *ACYRTHOSPIHON PISUM* (HARRIS) MANAGEMENT ON LENTIL, *LENS CULINARIS* (MEDIKUS)

by

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ABSTRACT

Damage caused by the Pea aphid, *Acyrthosiphon pisum* (Harris) is a limiting factor in lentil production in Ethiopia. Although application is minimal, losses are combated with the application of synthetic pesticides like Primicarb®. However, the continuous application of synthetic pesticides may result the development of insect resistance to insecticides, adverse effect on non-target organisms and environmental pollution. It is therefore necessary to implement a multi-faceted approach in order to keep *A. pisum* populations below economic threshold level.

This thesis highlights aspects of an integrated pest management approach to this pest. The components studied were host plant resistance, biological control and chemical control with bio-rational pesticides. All the trials were done under glasshouse condition in the University of the Free State, Bloemfontein, South Africa.

The host plant resistance study was completed in two phases. The first phase dealt with the preliminary screening of fifty entries of lentil introduced for such purposes. One entry appeared to be resistant while six were moderately resistant to *A. pisum.* The resistant entry and four of moderately resistant entries selected randomly were chosen for the next study. The second phase thoroughly examined and identified the mechanisms of resistance of each entry previously identified as moderately resistant and/or resistant.

With in the field of microbial control of agricultural pests, the effect of *Beauveria bassiana* on population of *A. pisum* was evaluated. This method appeared to be effective in significantly reducing the population of *A. pisum* compared with the control. The last component investigated the influence of the botanical product Neemolin® and extracts of Wild sering, *Burkea africana* on the fecundity of *A. pisum. A. pisum* populations treated with

Neemolin® produced significantly fewer offspring than the control and proved to be an effective control measure. On the other hand, application of extracts of Wild sering, *Burkea africana* did not affect *A. pisum* population. The result does not indicate the failure of this extract against the pest rather highlights the need to keep the extract in water for long hours so that the extract can dissolve and the insecticidal property can be enhanced.

The results of this study therefore indicate that the components of an integrated pest management approach included in this study will serve as a base towards effective management of this pest.

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CHAPTER 1

Introduction

Lentil, *Lens culinaris* Medikus is a high value profitable crop if properly managed. In 1998, world production was estimated at 2, 988,000 metric tones per annum. Ethiopia alone produced 37,000 metric tones per annum, which made her the top producing country in Africa. However, average yields have remained virtually static for various reasons.

Insect pests are a major factor, which limit productivity of lentils in Ethiopia. Foremost among the pests, is the pea aphid, *Acyrthosiphon pisum* (Harris) which sometimes causes total crop failures to lentils. Although the use of insecticides is limited, control measures rely on systemic insecticides like primicarb. Such dependence on pesticides is unlikely to be sustainable and results in considerable economic costs, ecological problems from pesticide resistance, and environmental concerns. In this scenario, the development of an IPM program thus becomes a high priority.

Any sustainable integrated pest management (IPM) system against *A. pisum* will require substantial input from the plant itself, biopesticides and biological control agents. Knowledge of the degree of cultivar susceptibility or resistance and the biology of the pest on a crop should be some of the key components of an IPM program for lentil. Thus, of central significance to an IPM program is knowledge of the degree of susceptibility or resistance of crop cultivars as well as how the effectiveness of control strategies can be influenced by the crop cultivar.

Naturally occurring epizootics of fungal diseases of *A. pisum* like *Beauveria bassiana* are ubiquitous reminders of the potential of this pathogen for control. Likewise, chemical control will remain a fundamental component of IPM, particularly with "bio-rational"

insecticides, as pesticides of plant origin are reasonably priced, readily available and cost effective in developing countries where synthetic pesticides are scarce and expensive for resource poor farmers. It was therefore, the goal of this study to make *A. pisum* management more efficient by investigating aspects of IPM.

CHAPTER 2

Production of lentil and biotic constraints with emphasis on pea aphid, *Acyrthosiphon pisum* (Homoptera: Aphididae)

2.1 Lentil, *Lens culinaris* (Medikus)

The two plant families of greatest importance to the world agriculture are the Poaceae (cereals and grasses) and the Fabacea (legumes). In terms of production volume, the cereals are the most important as they furnish the carbohydrates that constitute the major portion of human and animal diets (Hymowitz, 1990). Yet it is the family Fabaceae that shows most promise for producing the vastly increased supplies of vegetable protein that the world will need in the near future (NAS, 1979). In developing countries especially, cultivation of legumes is the best and quickest way to augment the production of food proteins (NAS, 1979). Their value lies in the nitrogen-rich plant material consumed by man and animals and the nitrogen-rich plant material they leave in the soil, thus enhancing the productivity of other crops grown in association with them (Polhill & Raven, 1981).

The Fabaceae has three sub-families, the Caesalpinioideae, the Minosoideae, and the Papilionideae; the first two consist mainly of tropical trees and shrubs with few economically important species. It is the Papilionideae that is of agricultural importance (Polhill & Raven, 1981).

The Papilionideae includes some 440 genera, consisting of 1200 species. These are further classified in 32 botanical tribes, six of which include the major vegetable and grain legumes. The Vicieae (Adans.) DC. includes the genera *Vicia* L., *Pisum* L. and *Lens* Mill.; the Cicereae Alefeld includes *Cicer*. The Phaseoleae DC. includes *Phaseolus* L., *Vigna* Savi, *Glycine* Wild and *Cajanus* DC. and the Aeschynomeneae (Benth.) Hutch, which contains

Arachis L. and the Genisteae (Adans) Benth. Which includes *Lupinus* L. and *Chamaecytisus* Link (Polhill & Raven, 1981).

Legumes are by far the most utilized plant family in terms of sheer numbers of genera and species used by humans (Hymowitz, 1990). The legume family contains about 650 genera and 18, 000 species. However, of the thousands of known legume species, less than 20 are used extensively at present (NAS, 1979).

Lentil is a short, slender, many-branched annual legume and generally has a bushy growth, which may range from fairly erect to more spreading in habit. Several workers (Zohary, 1972; Ladzinsky, 1993) reported that lentil was eventually placed in the genus *Lens* Miller after a confused and complex taxonomic history. The Latin name of the species, *Lens culinaris* was first published by Medikus in 1787 and predates the other common, but incorrect, name *Lens esculenta* that was published by Moench in 1794 (Webb & Hawtin, 1981).

The cultivated form is *Lens culinaris* Medikus ssp. *culinaris*. It is within the order Rosales, suborder Rosineae, family Fabaceae, subfamily Papilonaceae, and the tribe Vicieae. Four wild subspecies are recognized in the genus *Lens: L. orientalis, L. nigricans, L. ervoides, and L. odemensis.* Archaeological evidence, together with morphological and cytogenetic comparisons, suggest that *L. culinaris* was derived from *L. orientalis* (Zohary 1972, Ladzinsky 1993). Presently, it is known by many tribal names in different languages, e.g. adas (Arabic), masur (Hindi), mercimek (Turkish), heramame (Japanese) (Kay, 1979) and misir (in Ethiopia).

Lentil is a cool season legume species and as such is grown as a summer annual in temperate climates and as a winter annual in subtropical climates (Eriskine *et al.*, 1994).

According to Kay (1979), lentil requires environments ranging from cool temperate steppe to wet through subtropical dry to moist forest life zones. It is cultivated from sea level to 3,800m, but is not suited to humid tropics. Seeds require a minimum of 15^0C for germination, with an optimum of 18^0C - 21^0C (Duke, 1981). Good drainage is required; because even short periods of exposure to waterlogged or flooded field conditions kill plants (Oplinger *et al.*, 1990). Recently, Whitehead *et al.* (1998) reported that it is a remarkable and versatile crop, which can grow successfully in soil, which has poor nitrogen status, and in semi-arid conditions. There is no legume more resistant below 350 mm of precipitation and in the coldest climates: the lentil replaces all others in these conditions. It accompanies barley, which it leaves behind below 250 mm, when it is no longer possible to speak of agriculture in the strict sense (Hernando & Leon, 1994).

Lentil (*Lens culinaris* Medikus) is an important cool season food legume in Ethiopia occupying 50 000 hectares of land with a production of nearly 37, 000 metric tons. It plays a significant role in the diets of many people as a meat replacement during fasting days or seasons and as a cheap source of protein especially for low-income families. In their recent report on the Genetics and Breeding Research in lentil in Ethiopia, Bejiga & Anbesse (1994) stated that lentil is almost a cash crop because it fetches very high prices compared with all other food legumes and main cereal crops such as tef, wheat and barley. Cultivation of lentil in Ethiopia is generally limited to the highlands of Gonder, Semen Gojam and Shewa. The crop is sown with the onset of rains in June and harvested in October.

2.1.1 Origin and Historical perspectives

Lentil may have been one of the first agricultural crops grown more than 8,500 years ago (Oplinger *et al.*, 1990). It is one of Man's oldest food crops, originated in the Fertile

Crescent of the Near East, and dates back to the beginnings of agriculture itself. Lentil is mentioned in the Bible; the 'mess of pottage' made of red lentils for which Esau sold his birthright (Genesis 25). It is also listed in the Koran (Second Surah; Al-Baqarah) as one of the products of the earth which the Jews asked Moses to request from God, following the period in which manna and quails were the only food available to them (Webb & Hawtin, 1981).

According to Bahl & Sharma (1993), it is the oldest of the grain legumes to be domesticated. It is now cultivated in most subtropical and also in Northern Hemisphere such as Canada and Pacific Northwest regions (Muehlbauer & Abebe, 1997).

2.1.2 Distribution

From the near east and Mediterranean region, known to ancient Egypt and Greece, where it is still cultivated, lentil spread northward into Europe as far as the British Isles, east to India and much of China, and south to Ethiopia. It is now introduced and cultivated in most subtropical and warm temperate regions of the world, and high altitudes of the tropics, as well as Chile and Argentina (Duke, 1981).

The species is usually divided in to two main groups, namely *macrosperma*, which probably arose by selection from the *microsperma* (seed size ranging from 6 to 9 mm with red orange or yellow cotyledons). The former now predominates in southern Europe, North Africa, and North and Latin America. The *microsperma*, which are generally considered to be the older of the two groups, are now the main types cultivated in the Indian subcontinent, Afghanistan, Ethiopia and Egypt (Webb & Hawtin, 1981; Muehlbauer *et al.*, 1985).

2.1.3 Production

Table 2.1 shows the major lentil producing countries and the importance of this legume in these countries. During 1998, lentils were grown on about 3 400 000 ha, and total production was estimated at about 2 988 000 Metric tons. Average yields were just under 900 kg/ha.

In terms of production, Asia was the largest lentil-producing continent, and accounted for 74% of the total world production in 1998. The countries with the highest production were India, Turkey, Canada, Bangladesh, Syria, Iran, Nepal, China, U.S.A, and Australia, all of them, which produced more than 50 000 metric tons each in 1998. Yield varied widely from more than 1684kg/ha in Egypt to 436 kg/ha in Morocco by more than 3 times.

Ethiopia produced about 1.2% of the world's lentils (about 37 000 MT) and is the top producing country in Africa (Table 2.1). Most of the lentils are produced by low-income farmers who still practice traditional methods of cultivation. However, average production remained constant for four years and started to increase during 1999 (Fig. 2.1).

Lentil represents about 4.5% of the total world area sown to pulses (FAO, 1998). However, the area under lentil as a percentage of the total pulse area is much higher (Table 2.2) in certain individual countries, specifically in Asia. The highest is Nepal, where lentils comprise 53.6% of the total under pulses. Other countries with similarly high percentages include Syria (42.6%), Turkey (33.3%), Bangladesh (29.8%), Iran (24%) and Canada (23.8%). In Ethiopia, lentils comprise 4% of the total area under pulses.

Table 2.1 Area (000 HA) and production of lentil (000 MT) worldwide in 1998.

Continent/country	Area (1000HA)	Production (KG/HA)	Production (1000MT)
Africa	118	623	73
Ethiopia	51	725	37
Morocco	57	436	25
Egypt	4	1684	8
North & central America	445	1296	576
Canada	372	1291	480
USA	64	1370	88
South America	27	875	23
Argentina	10	1300	13
Asia	2690	817	2198
India	1200	736	883
Turkey	548	1069	586
Bangladesh	200	814	163
Iran	264	492	130
Nepal	155	732	114
China	90	1167	105
Syria	145	1074	156
Pakistan	65	571	37
Europe	42	721	31
Spain	27	591	16
Oceania	83	1040	86
Australia	82	1037	85
World	3404	878	2988

Source: FAO, 1998

Table 2.2 Total area under pulses and percentage of this under lentils in various countries and regions in 1998.

Continent/country	*Total area under pulses (000HA)*	*Percentage of total pulse area under lentils*
Africa	14252	0.8
Ethiopia	1290	4
Morocco	386	14.7
Egypt	193	2.1
North & central America	5448	8.2
Canada	1563	23.8
USA	970	6.6
South America	4333	0.6
Argentina	303	3.3
Asia	37200	7.2
India	24380	4.9
Turkey	1647	33.3
Bangladesh	671	29.8
Iran	1099	24
Nepal	289	53.6
China	3099	2.9
Syria	340	42.6
Pakistan	1728	3.8
Europe	4781	0.9
Spain	532	5.1
Oceania	2087	4
Australia	2061	4
World	68099	5

Source: Computed from FAO (1998).

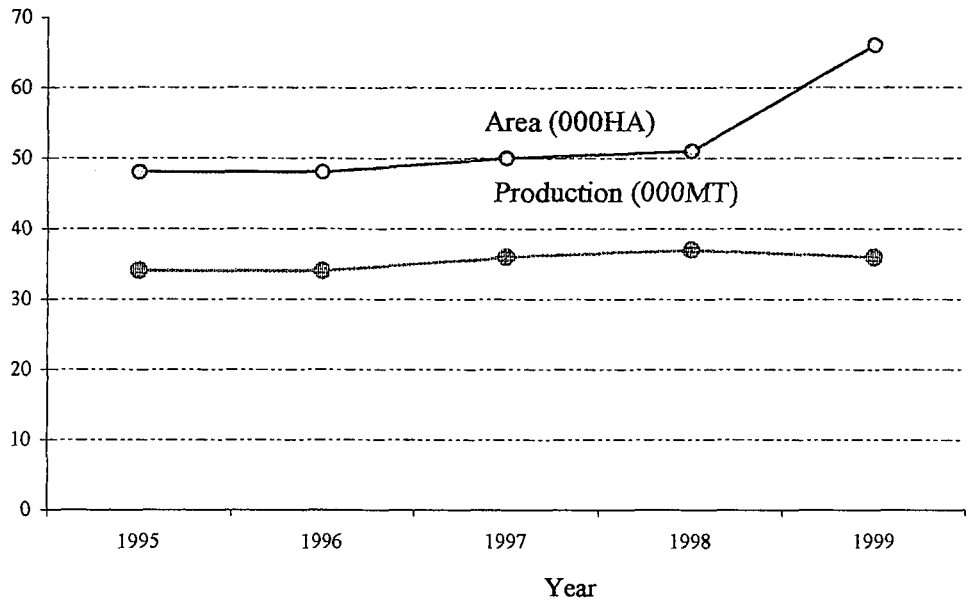

Fig. 2.1 Area under lentil and Production trend in Ethiopia from 1995 - 1999

2.1.4 Uses and nutritive value

Since the beginning of agriculture, grain legumes have had multiple uses depending on how the different parts of the plant were utilized. It is not surprising that grain legumes have had a favoured position in agriculture and in the human diet (Hernando & Leon, 1994). Among them, lentil is an excellent supplement to cereal grain diets because of its good protein or carbohydrate content (Oplinger *et al.*, 1990).

The primary product is the seed, which has a higher content of protein, carbohydrate and calories than other legumes (Table 2.3). It is the most desired crop because of its high average protein content and fast cooking characteristic in many lentil-producing regions (Muehlbauer *et al.*, 1985). Bressani & Elias (1988) reported that lentil seed has long been valued for its nutritional value, containing two to three times the protein concentration of cereals. Protein concentration of lentil ranges from 22-34.6% (Muehlbauer *et al.*, 1985; Oplinger *et al.*, 1990). It also has high carbohydrate concentrations (60%), mostly in the

form of starch, and the energy value per unit weight is equivalent to that of cereal grains (Bressani & Elias, 1988).

The seed can be consumed whole, decorticated, decorticated and split (usually the orange cotyledon *microsperma* are used for decortications) or ground in to flour (Nyaagard & Hawtin, 1981). It is used in soups, stews, casseroles and salad dishes (Oplinger *et al.*, 1990). Seeds can be fried and seasoned for consumption. Flour is used to make soups, stews, purees, and mixed with cereals to make bread and cakes; and as flour for infants (Williams & Singh, 1988). In certain countries, the young pods and leaves are used as a green vegetable and the sprouted seed may also be eaten (Nyaagard & Hawtin, 1981). A paste of cooked lentils was found in Egypt in a 12^{th} Dynasty tomb at Thebes (2400-2200 B. C.) and a fresco depicting the making of lentil soup dating back to the time of Pharoah Ramses III in 1200 B. C (Webb & Hawtin, 1981).

Legumes in general are consumed in one form or another in the everyday meals of Ethiopians. Traditional Ethiopian foods prepared from lentil include 'kik' (Dehulled split) consumed as a sauce/gravy, 'azifa' (cooked and mashed) consumed as a side dish and 'elbet' (paste from flour), which is eaten as a side dish (Yetneberk & Wondimu, 1994).

In developed countries, lentil is becoming a more popular food because of various health-related benefits. For example, saponin, a compound known to significantly reduce blood cholesterol concentrations, is present in relatively large concentrations in lentil seeds ($3.7\text{-}4.6 gkg^{-1}$) in comparison with cereal grains [e.g. $1.0 gkg^{-1}$ for oats (*Avena sativa*) [Fenwick & Oakenfull, 1983).

Unlike several other food legumes, few anti nutritional or toxic factors have been reported in lentils. They also require a comparatively short cooking time and are one of the

most easily digested of pulses (Nyaagard & Hawtin, 1981). Digestibility coefficients for lentil are relatively high and range from 78-93% (Hulse, 1990).

Lentil, which fails to meet food grade standards, can be used as livestock feed because of its high protein content and lack of digestive inhibitors (Oplinger *et al.*, 1990). Starch extracted from lentils has a stable viscosity over wide range temperatures and is sometimes used in the printing and textile industries (Kay, 1979; Nyaagard & Hawtin, 1981).

The medicinal properties of lentils have been mentioned in several old herbals. According to Van der Maesen (1972), chickpeas in the 6th century were believed to be an aphrodisiac; while curiously enough, lentils were considered to have the opposite effect, and this was probably the reason why the lentil was included in monasteries on meatless days. The 16th century writer Dondonaeus recommended them as part of the diet in monasteries as he believed they dampened the sexual appetite. Nicholas Culpepper, a noted 17th century astrologer/ physician, wrote that lentils were governed by the planet Venus. He went on to say that when eaten whole, with the seed coat, lentils 'bind the body and stop loosens the belly' (Nyaagard & Hawtin, 1981). Lentil is supposed to remedy constipation and other intestinal afflictions (Muehlbauer & Abebe, 1997). Other herbals report that lentils 'thicken the blood', which may relate to their comparatively high iron content (Nyaagard & Hawtin, 1981). Grain legumes also contain more dietary fiber than cereal grains (Abu-Shakra & Tannous, 1981).

As a food, lentils provide a valuable protein source, which, coupled with its ability to thrive on relatively poor soils and under adverse environmental conditions, has ensured its survival as a crop species to the present day (Webb & Hawtin, 1981). Thus, it appears to have been increasing slightly in importance in recent years. The wide range of uses to which

lentil, can be put, coupled with its value in many farming systems, is likely to ensure the continued cultivation of this crop (Nygaard & Hawtin, 1981).

Table 2.3 Estimated nutrient contents of lentils (g 100g seed^{-1}. i.e., %) compared with other important grain legumes.

Species	Calories	Water	Protein	Oil	Fiber	Carbohydrate	Ash
Chickpeas	358	11.0	20.1	4.5	4.9	56.6	2.9
Peas	346	11.0	22.5	1.8	5.5	53.7	5.5
Faba beans	348	11.0	23.4	2.0	7.8	52.4	3.4
Cowpeas	342	11.0	23.4	1.8	4.3	56.0	3.5
Lentils	346	11.0	24.2	1.8	1.8	59.0	2.2

Source: Muehlbauer *et al.* (1985)

2.1.5 Biotic constraints on the cultivation of lentil with special emphasis on Pea aphid

Grain legumes are important dietary constituents worldwide even though their overall production lags far behind that of cereals. Yields per unit area are generally less than one-half that of the major cereals. There are several reasons why grain legume yields in general and those of lentil (*Lens culinaris* Medikus) in particular are low (Muehlbauer *et al.*, 1985). These include insect pests and diseases.

2.1.5.1 Insect pests

Arthropod pests are one of the major constraints to agriculture production in Africa (Abate *et al.*, 2000). Insects are considered to be agricultural pests when numbers of a certain species increase to such a level that the yield of the marketable product is reduced causing economic losses.

Insects are not only responsible for direct production losses as a result of herbivory, but also cause massive indirect losses due to their role as vectors of various plant pathogens (Hilder & Boulter, 1999). Knowledge of the important injurious insects to a particular crop is increasingly important in agriculture. For some reason, the insect pests of lentils and other legumes have not received as much publicity as those, which infest cereals, however, they are just as important, especially in areas of food deficiency (Hariri, 1981).

Lentil crops are attacked by several pests wherever and whenever they are cultivated (Muehlbauer *et al.*, 1985). Field and storage insect pests of lentil reported around the world are listed in Table 2.4.

Several workers (Hawtin & Chancellor, 1979; Singh *et al.*, 1978) pointed out that the most commonly cited pests are pod borers (*Etiella zinckenella)*, aphids (*aphis* spp.), weevils (*Sitonia lineatus*), bruchids (*Bruchus* spp.) and cutworms (*Agrotis* spp.). The most important insects that damage pods and seeds are Lygus bugs (*Lygus* spp.), bruchid beetles (*Brucchus* and *Callosobruchus* spp.) and lepidoptera pod borers [*Helicoverpa armigera* (Hub), *Cydia nigricana* (F.), and *Etiella zinckenella* (Treitschke)] (Van Emden (1988)).

Bruchids not only destroy a large quantity of seed, but also reduce germination of seeds, which are damaged but not eaten completely. Several species are troublesome. The most important are *Bruchus lentis, B. ervi, and B. signaticornis* (especially in the Mediterranean region) and *Callosobruchus sinensis* (cosmopolitan). The degree of infestation can also depend on the cultivar, however, the variation in available germplasm is insufficient to warrant a breeding program for resistance (Muehlbauer *et al.*, 1985).

Infestation by *Sitonia* weevils (several species) can lead to economic losses in many regions. The larvae feed on roots and nodules and the adults damage leaves, producing a

typical crenellated margin. Soil-borne insects of occasional importance are cutworms (*Agrotis ipsilon*), bud weevils (*Apion* spp.), seed corn maggots (*Delia platura*) and wireworms (*Limonius* and *Ctenicera* spp.). Larvae of these insects destroy plants by feeding on stem apices of seedlings - a syndrome well known in other similar crops (Muehlbauer *et al.*, 1985).

Other insect pests known to cause economically significant damage include pea aphids (*Macrosiphon pisi*), cowpea aphids (*Aphis craccivora*) and thrips (*Megalurothrips* spp.) (Muehlbauer *et al.*, 1985). Aphids also transmit viruses from clover, alfalfa and other legumes growing near lentil fields. Pea enation mosaic virus, pea streak virus and various mosaic viruses are vectored in this way (Summerfield *et al.*, 1982).

Table 2.4 Field and storage insect pests recorded in lentil (*Lens culinaris* Medikus) producing countries around the world.

Country	Insect Pest	Reference
Argentina	Aphids (*A.Konodi*, & *A. Craccivora*); Thrips (*Caliothrips phaseoli*, *Frankliniella Schultzei* & *Sericothrips P.*	Manero & L' Argentier, 1987
Bangladesh	*C. chinensis*	Islam & Nargis, 1994
Czechoslovakia	Lentil gall midge (*Contarinia lentis*)	Kolesik & Sinsky, 1990
Egypt	Cowpea weevil (*C. maculatus* F.)	Nakhla, 1988
Ethiopia	*A. pisum; Aphis fabae* Scopoli; *Taeniothrips* spp.; *Epilachina* spp. & *C. chinensis.*	Ali & Habtewold, 1994
France	*A. pisum*	Rahbe *et al.*, 1995
India	*A. Craccivora* Koch.; *Bruchidae*; *C. chinensis* Linn.; Lentil pod borer (*Etiella Zinckenella* T.); *Heliothis armigera*	Eriskine *et al.*, 1994; Sharma *et al.*, 1991; Lal, 1992; Prasad, 1997; Ujagir, 1993
Pakistan	*A. Craccivora*	Solangi *et al.*, 1994
Spain	*Tychius quinquepunctatus* L.; *Bruchus lentis*	Monreal *et al.*, 1990
Syria	*Sitonia crinitus* H.	Weigand *et al.*, 1992
Turkey	*Amicta oberthuri*; *Heliothis viriplaca* (Hufn.)' *Bruchus lentis* Frohl.; *Sitonia crinitus* H.	Turkmen, 1987; Hincal & Kaya, 1988
USA	*A. pisum; Lygus hesperus; Sitonia lineatus; Thyanta pallidovirens* (Stal.)	Duke, 1981; Schotzko & O' Keffe, 1988; 1989; Anuj *et al.*, 1995; Kaiser *et al.*, 1993

2.2 Pea aphid, *Acyrthosiphon pisum* (Harris)

Aphids have a surprisingly long history and date back about 300 million years to the Mesozoic period (Adams & Van Emden, 1972). They are probably the most important and successful family of crop pests on a world scale, representing a vast and diverse assemblage of insects (Van Emden, 1972; Ishikawa, 1990; Campbell & Eikenbary, 1990). Among this assemblage are many of great economic importance by virtue of their detrimental effects to important crop or ornamental plants (Campbell & Eikenbary, 1990).

The largest families of aphids, the Aphididae, have achieved their success evolutionarily, and as agricultural pests, through parasitic exploitation of the temperate flora. The members of this flora make highly inconstant hosts, with marked seasonal cycles and a great diversity of growth patterns during the cool summer. A unique feature of aphids is that they have developed a specialized typically parasitic, "vegetative" mode of life without being committed to it, combining with it more normal locomotory, reproductive, and over wintering capacities within one species (Kennedy & Stroyan, 1959).

Their ability to avoid vacuole-sequestered toxins by moving their stylets intercellularly toward the relatively non-toxic sap flowing in the phloem, combined with parthenogenetic reproduction, has made this group one of the most successful groups of insects. This success has also made them one of the most devastating groups of pests of crop plants.

The pea aphid is a small (3 - 5 mg as an adult) (Fig. 2.2), oligophagous herbivore that feeds by removing sap from the vascular bundles of many legumes in the family Fabaceae (Mackay *et al.*, 1993). Adults and nymphs damage alfalfa by sucking sap from the various plant parts where they eventually cause yellowing, stunting and death of the plant (Painter,

1951; Shade & Kitch, 1983). The pea aphid, *Acyrthosiphon pisum* (Harris) also reduces stem elongation in these plants (Hutchins *et al.*, 1990). It feeds on plants by inserting a hollow stylet (Fig. 2.3) into the plant tissue to draw out the sap. In the United States, this pest causes millions of dollars of damage on alfalfa annually (App & Manglitz, 1972).

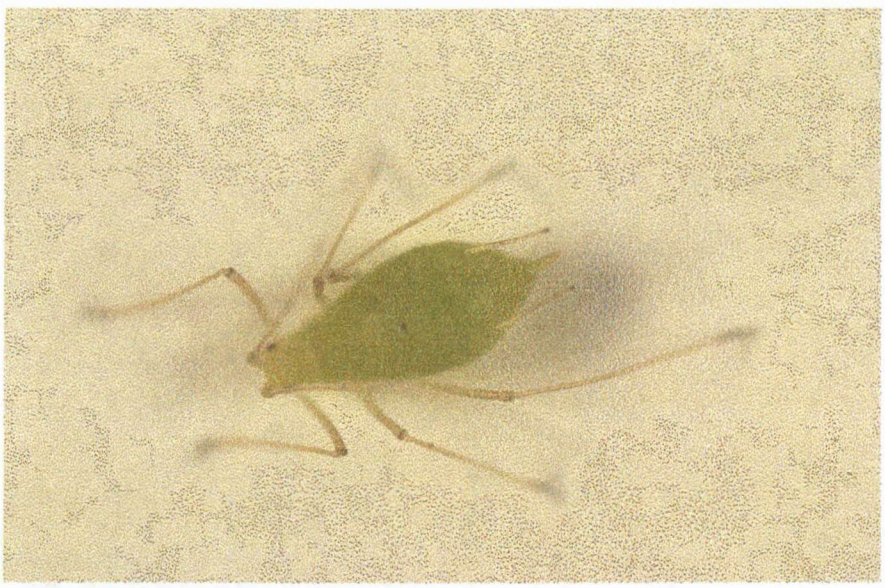

Fig. 2.2 Alate Pea aphid, *Acyrthosiphon pisum* (Harris)

Under laboratory conditions, pea aphid feeding reduced vegetative growth and nitrogen fixation of pea plants (Barlow *et al.*, 1977, Barlow & Messmer, 1982; Sirrur & Barlow, 1984). Maiteki & Lamb (1985) pointed out that high aphid densities reduced dry matter production, increased the number of pods per plant and the number of seeds per pod, increased the percentage of empty pods, reduced the seed weight and reduced the weight of nitrogen fixing nodules. They further stated that the correlation between aphid densities and the various yield components was higher for damage to young pods than to flowers or other pod stages, suggesting that young pods are very susceptible to direct feeding damage.

However, on forage alfalfa, pea aphid feeding did not reduce protein content (Cuperus *et al.*, 1982).

In Ethiopia, pea aphid occurs in all field pea-growing regions where infestation on local cultivars reaches 90-100% (Ali & Habtewold, 1994). They further explicated that farmers in different parts of the country ceased cultivation of field pea because this pest devastated the fields. This pest is also reported on lentil sometimes causing total crop failures. Infestation on lentil reaches 100% at peak flowering and at early pod setting of the crop (Ali & Habtewold, 1994).

Aphids are important vectors of plant diseases, particularly viruses. Pea aphid is a known vector of at least 25 different plant viruses, all but two being stylet-borne type (Avidov & Harpaz, 1969). Of these, lucerne mosaic, pea leaf roll, pea enation mosaic and pea mosaic virus in Great Britain, pea enation virus in the USA have been identified (McEwen *et al.*, 1957). In Israel, alfalfa mosaic virus, bean yellow mosaic virus, pea mosaic virus and pea enation mosaic viruses have been reported (Nitzany & Cohen, 1963).

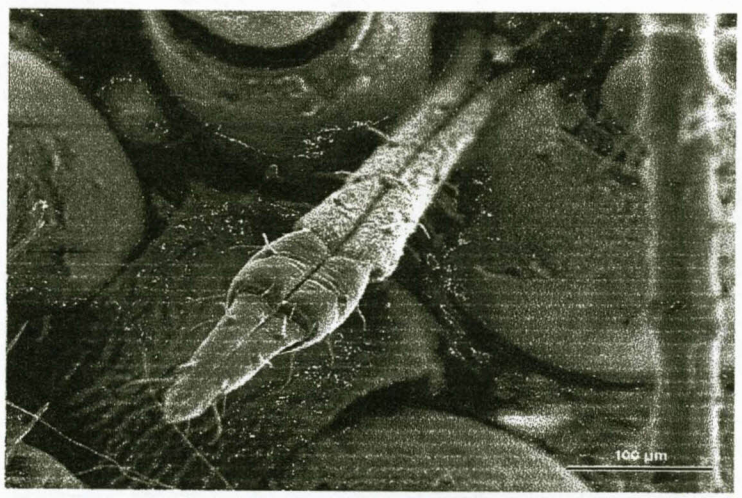

Fig. 2.3 Stylet of *A. pisum* used for sucking plant sap

2.2.1 Biology

Several workers (Kenten, 1955; Kennedy & Stroyan, 1959; Murdie, 1969a; b; Harison & Barlow, 1972; Frazer, 1972; Siddiqui & Barlow, 1973; Campbell & Mackauer, 1975; 1977; Hariri, 1981; Shade & Kitch, 1983; Lamb *et al.*, 1987; Damte, 1999) provided a detailed description of the biology of pea aphid. Lamb *et al.*, (1987) reported that the time from birth to the onset of reproduction was as short as 7 days, while 11 days has been reported by Frazer (1972) on *Vicia faba* L. A generation time of 6 days at 22^0 C has also been reported by Hochberg *et al.* (1986).

Glasshouse studies of Frazer (1972) indicated that the pea aphid had a reproductive period of 11 days, a post reproductive period of 6.2 days, fecundity of 96.7 offsprings and a generation time of 11 days on faba bean. In another study, Campbell & Mackauer (1975) observed the developmental time of two forms (apterae and alate) of pea aphid at four different temperature regimes. They noted that the duration of the nymphal stage was 7.6 days for apterous aphids and 8.2 days for alate aphids at 20^{O}C. At 14.8^{O}C, the developmental period extended to 12 and 13.9 days for apterae and alate pea aphids, respectively. The longest time recorded for the development of nymph at 10^{O}C was 23 days for apterae and 26.5 days for alate aphids. There was a decline in developmental time (5.4 days) with an increase in temperature (26^{O}C) for apterate aphids.

Under field conditions, Campbell & Mackauer (1977) reported that apterae 'Kamploos' biotype of pea aphid, *Acyrthosiphon pisum* required an average of 12.3 days or 134.0^{o}D above 5.56^{0}C to reach parturition. Prolonged exposure to high temperatures, such as 25^{0}C or above was detrimental to aphid development and survival (Harison & Barlow, 1972;

Kenten, 1955; Murdie, 1969a; b). The upper temperature limit estimated for pea aphid development is between 25°C and 30°C (Siddiqui & Barlow, 1973).

The nymphs molt four times and reach the adult stage and begin to reproduce in less than two weeks. Numbers can increase rapidly, as each female produces six or seven young per day until 50 to 100 nymphs are born during its life. In addition, there may be 20 or even more generations per season (Bolton, 1962; Hariri, 1981).

Morphologically, two distinct types of pea aphid are produced, alate (with wings) and apterae (without wings). The alate have specific adaptations for long distance dispersal (Mackay & Downer, 1979), and are known to travel long distances (Smith & Mackay, 1989). The production of alate is adaptively controlled by maternal age and environmental factors such as host plant condition and crowding (Sutherland, 1969a, b). Sexual females are always apterae, but the males may be apterae or alate (Smith & Mackay, 1989).

The existence of biotypes of pea aphid has been established. In his test of 31 lines from 4 geographical regions in the United States, Harrington (1943) was the first to recognize biotypes of the pea aphid based on host damage, size of the aphids and reproduction rates. Cartier (1959) discovered 3 biotypes in Quebec, Canada.

2.2.2 Host plants and distribution

As the genera of aphids tend to be associated with particular families of plants, so each species within an aphid genus tends to restrict its feeding to a certain genus or species of host plant, or at least to certain plant species within a clearly defined group of genera. The majority of pest aphids restrict their feeding to species within one plant family. However, they are able to colonize a wider range of alternate hosts, including economically important plants, than congeneric species (Blackman & Eastop, 1941).

Acyrthosiphon pisum, in the tribe Macrosiphini of the subfamily Aphidinae, exists as a number of races and subspecies with different host plant ranges and preferences (CAB, 2000). It infests a wide range of legumes including perennial and ornamental shrubs and annuals such as sweet pea (Daiber *et al.*, 1990). However, it is rarely found on non-legume hosts (CAB, 2000).

It is a widely distributed pest of many leguminous crops including peas, *Pisum sativum* (L.), alfalfa, *Medicago sativa* (L.), and lentils, *Lens culinaris* Medikus (Blackman & Eastop, 1984; Maiteki *et al.*, 1986); and also colonizes a few members of other tribes, e.g. Lotus (*Loteae*), Astragalus (*Galegeae*), and Glycine (*Phaseoleae*). Under dry conditions, it is sometimes found on *Capsella burapastoris* (Blackman & Eastop, 1984). According to the recent report of Bommarco & Ekbom (1996), *A. pisum* uses a wide variety of herbaceous legumes as host plants, and is not obliged to alternate between a winter and summer host. Secondary and wild hosts of this pest are given in Table 2.5.

A. pisum is probably of palaearctic origin (CAB, 2000), but now is virtually worldwide in distribution (Avidov & Harpaz, 1969, CAB, 2000). It was introduced into North America from Europe in the 1870's and spread rapidly across the continent as far as the arctic tundra. It was first recorded in South America in 1969 and in New Zealand in 1976 (Blackman & Eastop, 1984). The aphid invaded Australia as recently as 1982 and spread across that continent in 12-18 months after an accidental introduction (Davis, 1915; Dudley & Bronson, 1956; Mackay *et al.*, 1993) where it became a widespread pest of Lucerne (alfalfa), *Medicago sativa* L. (Milne, 1986). In Israel, pea aphid occurs all over the country (Avidov & Harpaz, 1969) while in Ethiopia, it occurs in all field pea and lentil growing regions (Ali & Habtewold, 1994). A list of countries with pea aphid is given in Table 2.6.

Table 2.5 Host plants of *Acyrthosiphon pisum* (Harris)

Secondary hosts	Wild hosts
Beta vulgaris var. saccharifera (sugarbeet)	*Astragalus* spp.;*Astragaus cicer* (Cicer milkvetch)
Carica papaya (Pawpaw)	*Capsella bursa-pastoris* (Shepherd's purse)
Cicer artienum (Chickpea)	*Cytisus* spp.; *Cytisus scoparius*
Cucumis sativus (Cucumber)	*Festuca arundinacea* (Reed fescue)
Curcurbita pepo (Ornamental gourd)	*Genista* spp.
Glycine max (Soya bean)	*Glycine* spp.
Lathyrus sativus (grass pea)	*Hippocerpis* spp.
Lens culinaris ssp. Culinaris (lentil)	*Indigofera hirsuta* (hairy indigo)
Onobrychis viciifolia (Sainfoin)	*Lathyrus* spp.
Vigna angularis (Adzuki bean)	*Lens* spp.
Vigna radiata (Mung bean)	*Lespedeza cuneata* (Sericea lespedeza)
Vigna mungo (Black gram)	*Lotus* spp.; *Lotus corniculatus* (bird's foot trefoli)
Phaseolus vulgaris (Kidney bean)	*Lupinus* spp.; *Lupinus ngustifolius; Lupinus luteus* (Yellow lupin)
Solanum tuberosum (Potato)	*Medicago* spp.
Spartium spp.	*Melilotus* spp.
Spinacia oleracea (Spinach)	*Melilotusofficiais* (Field melilot)
Trigonella foenum-graecum (Fenugreek)	*Onobrychis* spp.
Triticum aestivum (Wheat)	*Ononis* spp.
Vigna sesquipedalis	*Phaseolus* spp.
Vigna unguiculata (Cowpea)	*Pisum* spp.
Zea mays (Maize)	*Sarothamnus* spp.
	Sesbania spp.; *Sesbania exaltata*
	Solanum spp.
	Trigonella spp.
	Trifolium incarnatum (Crimson clover)
	Vicia spp.; *Vicia angustifolia* (Narrowleaf vetch); *Vicia villosa*
	Vigna (Cowpea)

Source: CAB, 2000.

Table 2.6 List of countries in which *A. pisum* has been reported.

Continent	**Country**
Africa	Algeria, Botswana, Burundi, Egypt, Ethiopia, Kenya, Libya, Malawi, Morocco, Rwanda, South Africa, Sudan, Tanzania, Uganda, Zambia and Zimbabwe
Asia	Afghanistan, China (6 states), Taiwan, Cyprus, Georgia republic, India (18 states), Iran, Israel, Japan (3 states), Jordan, Korea, Lebanon, Mongolia, Nepal, Pakistan, Philippines, Saudi Arabia, Syria, Thailand, Turkey, Uzbekistan, Yemen
Europe	Albania, Austria, Belgium, Bulgaria, Czech republic, Denmark, Faeroe islands, Finland, Former Yugoslavia, France (widespread), Corsica, Germany, Greece, Hungary, Iceland, Ireland, Italy, Luxembourg, Netherlands, Norway, Poland, Portugal, Madeira, Romania, Russian federation (2 states), Spain, Sweden, Switzerland, Ukraine, United Kingdom.
Western Hemisphere	Argentina, Bolivia, Brazil (4 states), Canada (9 states), Chile, El Salvador, Mexico, Peru, USA (in 49 states), Uruguay, Venezuela
Oceania	Australia (5 states), New Zealand

Source: CAB, 2000

2.2.3 Reproduction

Many aphid species are facultatively asexual, with parthenogenetic generations in the spring and summer alternating with one sexual generation in the autumn prior to the onset of the winter (Dixon, 1987).

Parthenogenetic reproduction evolved in aphids in the Permian, 200 million years ago, and has been of paramount importance in determining their population structure and high rates of increase (Dixon, 1987). Campbell & Eikenbary (1990) pointed out that parthenogenesis enabled aphids to telescope generations and achieve the prodigious rates of increase that are of great selective advantage when colonizing the temporarily un-exploited and highly favorable habitats available to all species of aphids at certain times of the year.

Pea aphid follows anholocyclic reproduction in warm countries. The alate and apterous females reproduce parthenogenetically through out the year. The population increases from February to November and declines during the winter. During the spring, it increases on the winter host plants. Winged viviparous females begin to spread to other leguminous host plants, including lentils, during April and May. These winged females give birth to young nymphs on the new host (Hariri, 1981).

In the Northern temperate parts of its range, the diapausing egg of the pea aphid hatches in the spring developing into a female called a fundatrix, which is characterized by short appendages (Mackay *et al.*, 1993). These females have shorter appendages and differ physiologically from later generations (Lees, 1960; 1966). The fundatrix is wingless and viviparous and reproduces parthenogenetically (Mackay *et al.*, 1993).

During the summer, viviparous parthenogenetic generations exhibit very high reproductive rates (Kennedy & Stroyan, 1959), 6-9 generations (Sandestrom, 1994). As the

summer ends and temperature and day length decline, the parthenogentic females give birth to sexual females and males (Lamb & pointing, 1972; Mackay, 1987). Lees (1966) reported that in climates where temperatures drop below freezing for extended periods and host plants become dormant, aphids such as *Acyrthosiphon pisum* (Harris) produce sexual morphs in response to shortening photoperiods and dropping temperatures. These mate and the females lay diapausing eggs that can withstand the winter (Mackay *et al.*, 1993). The sexual generation also provides genetic recombination and genetic variability that otherwise could only arise through mutation during the parthenogenetic part of the life history (Lynch, 1985).

In more temperate countries, the pea aphid hibernates as a diapausing egg on perennial legumes (Avidov & Harpaz, 1969). In Northern areas such as Canada, England, Finland and Sweden the pea aphid over winters exclusively as diapausing eggs (Bronson, 1935; Dunn & Wright, 1955; Markkula, 1963, Jones & Margaret, 1964; Sandestrom, 1994). In Central North America, eggs and asexual forms occur, but in the south only asexual forms are found (Jones & Margaret, 1964). Eggs are laid but the aphid may pass through very mild winters asexually (Dunn & Wright, 1955). In Australia, this aphid is thought to be anholocyclic and reproduce continuously on lucerne, which grows all year round there. However, at high altitudes in the snowy mountains of southeast Australia, the winters are sufficiently severe to prevent the aphids from surviving (Mackey *et al.*, 1989). Amphigonic reproduction is completely eliminated and the aphid reproduces exclusively by viviparous parthenogenesis in Israel, where the winter is much milder (Avidov & Harpaz, 1969).

Pea aphid does not reproduce sexually and has a life history indistinguishable from the summer individuals of facultatively parthenogenetic populations in part of its range where mild winters permit continuous growth and development (Mackay *et al.*, 1989). Many

aphid species that inhabit the subtropics, as well as some species in areas with more severe winters, continue to reproduce throughout the year if the winter is so mild that their host plants do not enter dormancy (Dixon, 1985). In these cases, sexual morphs and fundatrices are not usually found (Mackey *et al.*, 1989).

2.2.4 Control Methods

Various methods have been used to combat the damage caused by aphids. These include the unilateral investigations on host plant resistance, biological, cultural, and chemical control methods and more interestingly the amalgamation of one or more control methods presently known as 'IPM'.

2.2.4.1 Host Plant Resistance

One of the mainstays of integrated pest management is the use of crop varieties that are resistant or tolerant to insect pests. A resistant variety can be less preferred by the insect pest (antixenosis), adversely affects its normal development and survival (antibiosis), or the plant may tolerate the damage with out an economic loss in yield or quality (tolerance). The development of resistant or pest - tolerant crop varieties, however, may require considerable time and money, and resistance is not necessarily permanent. Just as insect populations have developed resistance to insecticides, populations of insects have developed that are now able to cause concomitant damage to plant varieties that were previously resistant.

Host plant resistance has often been used alone in pest management systems, but the benefits of resistance depend on type, level, and crop production system under consideration. The use of host plant resistance as the key component of an IPM system has greater potential than any other tactic for pest suppression, as plant resistance is specific to a key insect or

insects, has cumulative effectiveness, is persistent, compatible with other IPM techniques, environmentally friendly, and is easily incorporated in to a normal farm operation (Panada & Khush, 1995).

The earliest documentation on host plant resistance dates back to 1782, when a wheat variety resistant to Hessian fly was reported by Havens (Panada & Khush, 1995). Much of the impetus to investigate sources of resistance in plants to insect attack came from Painter and his colleagues in Kansas, who were concerned with selecting resistant genotypes of crop plants and with breeding programs for the development of resistant commercial varieties.

Insect resistant plants provide ideal solution for the control of insects because it is effective and economical, it avoids insecticide hazards, and the protection usually lasts for many years (Gorz *et al.*, 1979). An improved understanding of host plant resistance requires (Robinson, 1993) that the modes of resistance of host plants are defined. Painter (1951) describes the resistance mechanisms as non-preference, tolerance and antibiosis. However, the term non-preference has subsequently been replaced by antixenosis (Kogan & Ortman, 1978). According to Painter (1951) preference or non-preference denotes the group of plant characters and insect responses that attract or repel from the use of a particular plant or variety, for oviposition, food, shelter, or for combinations of the three. Antibiosis represents the tendency to prevent, injure, or destroy (insect) life. The effect on the insect takes the form of reduced fecundity, decreased size, abnormal length of life and increased mortality. He described tolerance as a form of resistance in which the plant grows and reproduces itself to repair injury in spite of supporting an insect pest population approximately equal to one that is damaging to a susceptible host.

To date resistant varieties have been identified for various crops. Resistance to the pea aphid has been reported in garden peas (Searls, 1935; Maltais, 1936; Harrington, 1941); in alfalfa (Blanchard & Dudley, 1934; Dahms & Painter, 1940) and in red clover (Wilcoxon & Peterson, 1960; Markkula & Roukka, 1970). Varying differences in resistance to pea aphid damage has also been reported among cultivars and crops at different times.

In this respect, Markkula & Roukka (1970) studied the resistance of 10 red clover varieties to pea aphid biotypes 1a, 1b and 16. They noticed that all the clover varieties were resistant to biotype 1a, the number of progeny on them being less than 10 while only some of them proved to be resistant to biotype b. However, all of the varieties were susceptible to biotype 16. Early findings of Wilcoxon & Peterson (1960) indicate that the Dollard variety of red cover was more resistant than the Wegener variety in terms of aphid reproduction.

Very recently, Bournoville *et al.* (1999) evaluated a susceptible line of alfalfa [lucerne] (*cv. Milfeuil*) for resistance to the pea aphid (*Acyrthosiphon pisum*), using a tolerance test of seedlings to an infestation with a fixed biomass of aphids. When comparing the first and the fourth generation of selection for seedling tolerance, they reported an increase in antibiosis. In another study on the modalities of resistance on three clover varieties, Zeng *et al.* (1994) reported that survival and fecundity were significantly lower for *A. pisum* reared on N-2 than for those on one or both of the susceptible clovers ('Tensas' and 'Redland') in a no-choice experiment. They further stated that the resistance operating in N-2 was a combination of antixenosis and antibiosis. Antibiosis has been reported to be part of the resistance phenomena in *Macrosipum pisi* (Klitb.), pea aphid on peas (Harrington, 1941).

In their study Dreyer *et al.* (1987) mentioned that among the features that deter the development of pea aphid on resistant alfalfa, the rate of enzymatic-catalyzed

depolymerization of the pectin isolated from different alfalfa lines correlates with plant resistance to aphids. However, Rahbe *et al.* (1988) argue that the role of certain secondary metabolic products in host-plant resistance to aphids cannot just be dismissed out of hand.

Advances in plant biotechnology also provided management of insect pests through genetic engineering. With the advent of genetic transformation techniques, it has become possible to clone and insert genes in to the crop plants to confer resistance to insect pests. Resistance to insects has been demonstrated in transgenic plants expressing genes for δ-endotoxins from *Bacillus thurgiensis* (Bt), Protease inhibitors, enzymes and plant lectins (Sharma *et al.*, 2000).

Considerable progress has been made in developing transgenic crops with resistance to the target pest over the past decade (Hilder & Boulter, 1999). Genes conferring resistance to insects have been inserted in to crop plants such as maize, cotton, potato, tobacco, rice, broccoli, lettuce, walnuts, apples, alfalfa and soyabean (Bennet, 1994; Federci, 1998; Griffiths, 1998). Such transgenic plants have shown good promise in reducing insect damage, both in the laboratory and field conditions (Sharma *et al.*, 2000). There is a also need to use these tools for providing resistance in insects in cereals, legumes and oil seed crops that are a source of sustenance for poorer sections of the society (Sharma *et al.*, 2000).

Transgenic plants with insecticidal genes are set to feature prominently in pest management in both developed and the developing world in future. Among the developing countries: China, India, Argentina, Mexico, Brazil, Pakistan, and South Africa are pursuing the research on transgenic crops vigorously (Sharma *et al.*, 2000). Though many shortcomings have been reported on transgenic crops, the selection and incorporation of resistant genes in to crop plants continues to be effective means of combating pest damage.

However, host plant resistance alone cannot be the permanent solution because insects also develop new biotypes that can overcome the resistant gene with in the crop.

2.2.4.2 Biological Control

Attempts to use entomopathogenic fungi as inundative control agents of insects began in the late 1800's (Mc Coy *et al.*, 1988). However, much impetus was lost with the advent of effective chemical pesticides (Lacey & Goettel, 1995). Interest was revived in the 1960's and several products based on *Beauveria bassiana* for the control of numerous pests in the People's Republic of China (Feng *et al.*, 1994) and the Colorado potato beetle, *Leptinotarsa decemliniata* (Say), in the former USSR (Ferron 1981).

The importance of entomopathogenic fungi as biological control agents has been reviewed by Latge & Moletta (1988); Mc Coy *et al.*, (1988); Mc Coy (1990); Ferron *et al.*, (1991); Roberts & Hajek (1992); Tanada & Kaya (1993); and Hajek & St Legar (1994). The majority of entomopathogenic species are classified in the classes *Hyphomycetes, Zygomycetes* (order *Entomophtorales*) and Ascomycetes (in particular, the genera Cordyceps and Torubiella) (Gillespie & Moorhouse, 1989).

Entomopathogenic fungi can be placed in to two broad categories in respect to potential safety. Highly specific fungi, which putatively pose a minimal threat to invertebrate non-target organisms, include species such as *Aschersonia aleyrodis.* These are restricted to several homopteran, and Lepidopteran species; *Panadora neoaphidis*, restricted to aphids; and *Entomophaga grylli*, which are restricted to Orthoptera (MacLeod, 1963). Other fungi with very wide host ranges include *Beauveria bassiana, Metarhizium anisopliae, Paecilomyces* and *Zoophthora radicansand* (Goettel, 1995). This has raised concerns over safety. For instance *Beauveria bassiana* has been recorded infecting over 700 species of

arthropods (Li, 1988). However, fungi with wide host ranges are prime candidates for inundative control (Goettel, 1995).

Several species of entomopathogenic fungi can cause fatal disease in aphids, including *Verticillium lecanii* (Zimmerman, 1986), various species of *Beauveria*, and *Paecilomyces farinosus* (Roberts & Yendol, 1971; Samson *et al.*, 1988). Many species of aphids are controlled by the natural occurrence of the fungus genus Entomophtora (Shands *et al.*, 1972). He mentioned that Entomophtora infections were probably the major factors in controlling the green peach aphid, *Myzus persicae* (Suzler), in Maine.

Insect pathogenic fungi are considered by some as promising natural enemies for applied aphid biological control. However, few are capable of causing high mortality in aphid populations (Latge & Papierok, 1988; Milner, 1997). Apparently no epizootics caused by bacteria, viruses, protozoa, or nematodes have been reported from aphids (Hagen & Van den Bosch, 1968).

The first microorganism to be recognized as a disease agent was the fungus *Beauveria bassiana* (Bassi, 1835). The genus *Beauveria* has been monographed by MacLeod (1954), who recognized two species, *B. bassiana* and *B. brongniartii* that attack all stages of insects of all groups. *Beauveria bassiana* is an important insect pathogen that infects a wide array of insect hosts (Hall 1976, 1981; Ferron 1978, 1981; 1991; Goettel 1992; McCoy *et al.*, 1988; Tanada & Kaya, 1993; Zimmermann, 1986). It has one of the largest host lists among the imperfect fungi and occurs in soil as a ubiquitous saprophyte (Mc Coy *et al.* 1988; Tanda & Kaya, 1993). *Beauveria bassiana* was recovered from the pea aphid, *Acyrthosiphon pisum* (Harris), by Pavliushin (1983) in USSR (Feng *et al.*, 1990). This species generally infects through the integument (Cheung & Grula, 1982).

Despite its advantages, studies relating to the use of *Beauveria* spp. for aphid control are in the preliminary stages (Brown & Smith, 1974). Its use in agricultural pest control has been limited due, in part, to inconsistent results obtained from field applications (Ferron, 1981). Variation in degree of pest control can be caused by numerous factors that are capable of limiting the effectiveness of fungal pathogens. Among these are difficulties in preparing and applying fungal formulations, short storage life, short life on plant surfaces, and the requirement of high relative humidity for a prolonged period to start conidial germination (Ferron, 1981). The effect of some of these could be reduced by the use of specific, improved formulations of fungal material for field application (Knudsen *et al.*, 1990). One of the most common methods to increase or regain lost virulence is to pass the entomopathogenic fungus through a living insect. For example, increased virulence in *Beauveria bassiana* (Balsamo) Vuilmerin can be obtained by passaging the fungus through pea aphid (Aizawa, 1971).

The pest status of some aphids such as *Acyrthosiphon pisum* is considerably reduced by natural epizootics of fungal disease. However, disease may contribute little to practical control, as is mainly effective in high-density populations when weather conditions are suitable (Milner, 1997). Although the factors that initiate the occurrences of Entomophtora infections in aphids are largely environmental and unpredictable, once Entomophtora infections appear in dense aphid populations, epizootics usually occurs making further control measures unnecessary (Maddox, 1975).

However, recent reports of James *et al.* (1995) indicate that pea aphid, *Acyrthosiphon pisum* populations were not affected by one aphid - derived strain of *Beauveria bassiana* under field conditions. However, they reported that fungal conidia persisted in the field for at least 28 days, when approximately 10% of the original inoculum was still present.

In as much as pathogens are closely associated with parasites and predators in nature, and are generally compatible with the most chemical insecticides, they afford comprehensive opportunities in integrated control of insect pests. However, dependence on biological control alone does not offer an entirely satisfactory option.

2.2.4.3 Cultural Control

Cultural control implies practices that make the environment less attractive to pests and less favorable for their survival, dispersal, growth and reproduction, and that promote the pest's natural controls. The use and manipulation of cultural practices is the oldest method that has been used to manage pest populations. Many of the systems have become traditional eliminating the need for high levels of knowledge. In the case of commercial agriculture, many of these practices are too labor intensive for use in large-scale monoculture.

Several workers reported the effectiveness of cultural control against aphid populations. In 1989, Lal *et al.* noted that planting density influenced aphid numbers. He indicated that fewer black aphids were on chickpea plants sown 30 cm X 10 cm apart than on plants sown 60 cm X 20 cm apart, irrespective of the cultivar sown. There is also considerable literature that indicates close spacing reduces aphid infestation in beans (Ogenga-Latigo *et al.*, 1992a, b).

On the contrary, Furuta & Aloo (1994) noted that increased spacing prevented or delayed the spread of Sakhalin fir aphid, *Cinara todocola* Inouye, and decreased the percentage of trees infested in Japan. They further stated that more widely spaced planting might be an alternative to parasitoid release in the integrated control system. In Poland, Wnuk & Wiech (1996) reported that increasing the spacing between pea plants decreased the number of aphids (*Acyrthosiphon pisum*).

In the field, beans inter-cropped with densely planted maize suffered reduced *A. fabae* attack, particularly when inter-cropped with old maize crops. On the other hand, in glasshouse tests with alates of *A. fabae,* few aphids penetrated the maize canopy to reach and colonize plants of *P. vulgaris* when maize plants were densely planted (Ogenga-Latego *et al.*, 1992a).

2.2.4.4 Chemical control

Despite extensive attempts after the Second World War to reduce pest attacks using numerous synthetic pesticides, insects remain the main competitors of man for food, especially in developing countries. Owing to indiscriminate use of pesticides, various side effects have been observed in man and the environment, and many insect pests have become resistant to one or more pesticides (Schmutterer, 1988).

The use of synthetic pesticides in agriculture during the last 45 years has played an essential role in the production of an abundant food supply (Stark *et al.*, 1992). However, use of some pesticides has resulted in environmental contamination (Frank *et al.*, 1990), negative effects on non-target organisms (Bender, 1969; Mulla & Mian, 1981; Gary & Mussen, 1984), and the development of resistance (Brattsen *et al.*, 1986, Tabashnik *et al.*, 1987). Therefore, they do not fulfill the requirements of integrated pest management (IPM) unless used judiciously. For this reason as well as the increasing problems of pest resistance to pesticides interest in insecticidal botanicals has grown rapidly during recent years (Schmutterer, 1990).

For centuries humans have used natural insecticides to combat insect pests that compete for our food and fiber or that affect public health (Coats, 1994). Among the numerous ingredients of plants studied during the last 20 years, extracts and compounds from

the neem tree, *Azadirachta indica* A. Juss, have attracted the interest of entomologists and phytochemists all over the world (Schmutterer, 1990).

Azadirachta indica (syn. *Antelaea azadirachta, Melia azadirachta*) is a tree belonging to the Meliaceae (mahogony) family. It is an evergreen, or deciduous fast-growing plant, which may reach a height of 25 meters. It thrives primarily in tropical climates that have an annual rainfall of 400 to 800 mm and an extended dry season (Schmutterer, 1990). This tree can tolerate severe droughts, poor, shallow and even saline soils (Radwanski *et al.*, 1981).

Native to the Indian subcontinent, this fast growing shade tree has been widely cultivated in Africa, Australia, the Caribbean, and Central and South America. Although the seeds and leaves of this tree have been traditionally used for centuries to control pests (Koul *et al.* 1990), recent interest in neem as a crop protectant dates back to the work of Pradhan *et al.* (1962), who reported that dilute seed extracts completely prevented feeding of the desert locust, *Schistocerca gregaria.*

According to Schmutterer (1990), fruits are the most important source of the ingredient of neem, which affect insects in various ways. The major active principle, azadirachtin (AZA), a ring C-seco tetranortriterpenoid, is the most potent natural insect antifeedant discovered to date (Isman *et al.*, 1990). Its insecticidal properties have been extensively investigated in recent years. Activity against >200 species of insects (Isman *et al.*, 1990) has been reported. However, the quality of this compound may vary considerably because of environmental factors and possibly also for genetic reasons (Schmutterer, 1990).

The diverse biological activities of neem or Azadirachtin include feeding and ovipositional deterrence, repellency, growth disruption, reduced fitness and sterility (Koul *et al.*, 1990; Schmutterer, 1988; 1990).

The antifeedant action of neem is documented by many workers. A reduction in food intake of *N. lugens* has been reported by Saxena *et al.* (1984) on caged rice plants grown in neem cake-incorporated soil. In Orthoptera, the "primary" (gustatory) antifeedant effect seems to be of special importance. A number of locust and grasshopper species refuse to feed on neem-treated plants for up to several days, sometimes for a longer-period; these include the desert locust, *Schistocerca gregaria* (Schmutterer, 1990). This pest prefers to die from starvation than to feed on treated food plants (Schmutterer, 1988).

More recently, Azadirachtin has been demonstrated to strongly interfere with moulting and reproduction in several species of insects (Koul *et al.*, 1987; Siber & Rembold, 1983), which points to the neuroendocrine system as a target site.

Most studies on neem have involved insects with chewing mouthparts; where as agricultural pests with piercing and sucking mouthparts have not been thoroughly investigated (Schmutterer, 1990). Aphids are economically important pests that are difficult to control because of their mobility, tremendous reproductive ability, and resistance to many synthetic pesticides (Van Lenteren 1990). Studies indicated that neem based insecticides can be effective in controlling aphids and might be suitable for inclusion in integrated pest management programs (Schmutterer, 1988).

The effect of neem on aphids is well documented. Aphids exposed to neem seed oil or Azadirachtin produced large numbers of dead offspring, most likely caused by an inhibition of cuticulogenesis and ecdysis, as has been shown to occur during the development of nymphal *Rhodinus prolixus* Stal and *Locusta migratoria* L (Garcia & Rembold, 1984; Sieber & Rembold, 1983). Lowery & Isman (1996) indicated that azadirachtin had a greater negative impact on the fecundity of lettuce aphid, *Nasonovia ribisinigir* (Mosley) exposed as

4^{th} instars compared with treatment of adults. Because ovulation occurs throughout the nymphal development of aphids (paedogenesis) and 4^{th} instars contain embryos that have begun primary oocyte development (Blackman, 1978), azadirachtin would likely exert a stronger sterilizing effect following treatment of neonates as compared with adults. In addition neonates are more sensitive to the sterilizing action of neem because of their smaller size.

A. pisum, placed as 1^{st} instars on broad bean, *Vicia faba* L., sprayed with a 0.002% methanolic neem seed extract produced 1.6 offspring per female per day, compared with 7.1 offspring per female per day in the controls (Schauer, 1985). In another study, Schauer (1984) noted that neem seed kernel extracts applied to broad bean in the laboratory killed first-instar pea aphids, *Acyrthosiphon pisum* Harris, and black bean aphids, *Aphis fabae*. Recent investigation of Lowery & Isman (1996) also indicated that neem seed oil and Azadirachtin effectively inhibited aphid reproduction. Exposure to Azadirachtin also resulted in fewer developing embryos, most likely caused by reduced oocyte maturation as has been shown for other insects. In addition, azadirachtin might delay the growth of aphid embryos, which would help explain how azadirachtin rapidly inhibits the reproduction of aphids that contain embryos at various stages of development.

Control of aphids in the field with foliar applications of neem would result from the combined negative effects of azadirachtin on aphid reproduction and survival (Lowery & Isman, 1993). Because of a tremendous reproductive ability of aphids, a decrease in fertility would enhance the control of aphids by natural enemies (Lowery & Isman, 1996).

Although neem products have been shown to act against such a large number of insect species, they apparently do not kill many of the beneficial insects, such as the

predators and parasitoids. Tests on non target arthropods, fish and livestock have indicated excellent selectivity and residues in the environment are short-lived, especially in sunlight (Saxena, 1989).

2.2.4.5 IPM

The unilateral use of any control method can have unwanted and unintended side effects. The application of a chemical to destroy an insect pest, or the planting of an insect resistant variety of crop plant, or even the introduction of a new biological agent to control a pest, may have a dramatic impact on other aspects of the ecosystem.

The idea of modifying chemical and cultural control measures so that they work with rather than against natural enemies of a pest is not new. It has developed, largely as a result of the undesirable side effects of the use and misuse of the newer persistent organochlorine insecticides (Jones & Margaret, 1964). A burgeoning literature on integrated pest management (IPM) has arisen since 1959 when the term Integrated Pest Control was defined (Stern *et al.*, 1959). The history of IPM, however, can be traced back to the late 1800's when ecology was identified as the foundation of scientific plant protection (Kogan, 1998).

IPM practices involving combinations of physical, biological and chemical controls were recommended as early as 1860 in a classic book on farm insects (Curtis, 1860). Kogan (1998) pointed out the recognition of the failings of the new organosynthetic insecticides, namely resistance, resurgence of primary pests, upsurges of secondary pests, and overall environmental contamination allowed the growing popularity of the integrated control concept.

The fundamental importance of IPM is evidenced in its recent adoption as a basic tenet of the sustainable agriculture movement. However, integrated control only achieves this

ideal by harmonizing techniques in an organized way, by making the techniques compatible, and by blending them in to a multifaceted, flexible system.

Integration of cultural practices to encourage beneficial species is sometimes relatively easy (e.g. retention of stubble containing parasitized over wintering insects). Integration of chemical measures is only possible when a pesticide can be used selectively to kill a pest but leave relatively untouched one or more of its effective natural enemies. Nicotine to control aphids was used in this way. Most of the aphids were killed, while parasites and many predators were unharmed and the surviving aphids were soon destroyed (Jones & Margaret, 1964).

Agricultural scientists in national and provincial agricultural research systems have been actively engaged in various aspects of pest management in Africa since the beginning of 1970s. Abate *et al.* (2000), stated that of a total of 51 IPM or IPM related projects had been initiated between 1972 and 1992. About 34% dealt with biological control using natural enemies, 27% with chemical control, 15% with scouting and monitoring, 13% with host plant resistance, and 11% with cultural practices.

A detailed review of IPM in Ethiopia has been made by Abate (1995). As in many countries in Africa, control of crop pests in Ethiopia is achieved through the use of a traditional IPM approach that consists of appropriate cultural practices, varietal resistance, and use of locally available materials (Abate & Ampofo, 1996). Successful IPM programs include those for the pink bollworm *(Pectinophora gossypiella),* which was a major pest of cotton in Ethiopia during the early 1970s. This included the use of a "closed" season, coupled with the collection and destruction of larvae on a trap crop (cotton) planted in mid-April, which has successfully kept the pest population below economic level (Abate, 1982). Results

of studies on the biology and cultural practices have also helped to reduce the frequency of insecticide sprays on other bollworm species *(Helicoverpa armigera, Earias biplaga, E. insulana, Diparopsis watersi)*. Another successful example is an IPM program, which was based on understanding the population dynamics of the pest and its natural enemies, and identification of selective insecticides against red scale *(Aonidiella aurantii)*. This species was the major pest of citrus orchards in the state farms during the late 1970s, but the pest is no longer a great threat to the citrus industry of Ethiopia.

Ideally, for resource-poor farming, pest management should require no extra cash from the farmer, as exemplified by classical biological control against accidentally introduced exotic pests or the yet to be realized potential for use of exotic natural enemies against indigenous pests. The crucial need, therefore, is to make the low input agriculture more sustainable. Otherwise IPM inputs may be irrelevant.

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CHAPTER 3

Evaluation of lentil genotypes for resistance to the pea aphid, *Acyrthosiphon pisum* (Harris) (Homoptera: Aphididae)

Abstract

Fifty entries of lentil were evaluated for resistance to *Acyrthosiphon pisum* under glasshouse conditions. Two weeks old lentil seedlings of each entry were infested with five fourth instar *A.pisum* from a stock colony. The number of progeny was recorded 14 days after infestation. Analysis of count data indicated that the final number of aphids differed among entries ($P < 0.05$), and ranged from 196 nymphs on the susceptible line, ILL 8127, to 16 nymphs on the resistant entry 'Verdina'.

Key words: Lentil, *A. pisum*, resistance

3.1 Introduction

The pea aphid, *Acyrthosiphon pisum* (Harris) is a widely distributed pest of many leguminous crops including peas, *Pisum sativum* (L), alfalfa (*Medicago sativa* (L), and lentils, *Lens culinaris* Medikus (Blackman & Eastop, 1984; Maiteki *et al.*, 1986). It feeds on the plant by inserting a hollow stylet in to the plant tissue to draw out the sap from the vascular bundles of many legumes. The damage is usually expressed as yellowing, stunting and death of the plant. Repeated applications of aphicides are therefore necessary to protect lentils from infestation.

In Ethiopia, insecticide use has been the principal control strategy against *A. pisum* on lentil and field pea. However, due to the deleterious effects of synthetic insecticides on man, and the environment, research over the years has focused mainly on biologically based management methods, such as the development of aphid-resistant cultivars. Perhaps even more importantly, as an integral component of integrated pest management, the development and utilization of varietal resistance in the management of this pest has great economic potential, as it offers ecological advantages compared with use of insecticides and involves no extra cost to the farmer.

To date various pea aphid resistant cultivars of a variety of legume crops have been identified. However, with increasing use of plant resistance and transgenic crops, there is an increasing probability of the development of new pea aphid biotypes that can overcome resistance. Therefore, the continuous selection and use of pea aphid resistant genotypes is crucial. However, the reluctance of farmers accepting insect resistant genotypes in preference to a traditional, susceptible cultivar remains a problem. During this study, fifty genotypes of lentil from different parts of the world were screened for resistance to the pea aphid.

3.2 Materials and Methods

Aphid colony: Technique for rearing pea aphids was adapted from procedures described by (Campbell & Mackuer, 1975). Pea aphid colonies were cultured in greenhouse on a susceptible Ethiopian field pea variety, 'Mohanderfer', from parthenogenetic females collected from the Agronomy farm of the University of Free State. When reproduction began, the newborn aphids were transferred to lentil seedlings. As plants deteriorated, they were replaced with new ones and the aphids were transferred to them. The continual introduction of fresh plants maintained aphids in good condition. The stock culture was maintained on potted plants of lentil in a glasshouse insectary at 20 ± 2^{0}C and 16:8 (L: D) hr photoperiod supplemented with artificial lighting. At least one pea aphid generation was completed on lentil before use in the experiment.

Plants: Fifty lentil entries introduced for screening purposes were each planted (3 seeds per pot) in 1lit plastic pot filled with standard greenhouse soil. The experiment was arranged as a randomized complete block with three replications. Seedlings were then thinned to one after emergence. Test entries were selected based on the information on their yield potential.

Infestation: When plants were two weeks old, each pot was infested with five 4^{th}-instar pea aphids. Aphids were placed on each plant using fine camel's hair brush, and then caged in ventilated nylon organdy, supported by a steel stand, which fitted tightly over the pots to circumvent aphid escape (Fig. 3.1). Aphid-infested plants were watered every other day and were maintained in the greenhouse at 20 ± 2^{0}C with a photophase of 16:8 (L: D) hr supplemented with artificial light (Frazer, 1972).

Data collection and analysis: The number of aphids on each lentil entry was recorded 14 days after infestation. These counts were square root transformed to normalize the distribution and stabilize the variances. Duncan's multiple range test at a probability level of $P < 0.05$ was used to compare means (MSTAT-C, 1990). During mean comparison, back transformed data was used. Finally, entries were categorized in to resistance ratings according to modified scoring scale of Ellusbury *et al.* (1985) where 1= no aphids (Immune); 2 = 1-10 aphids (Highly resistant); 3 = 11-20 aphids (Resistant); 4 = 21-50 aphids (Moderately resistant); 5 = 51-100 aphids (Moderately susceptible) and 6 = > 100 aphids (Susceptible).

Fig. 3.1 The experiment during the study period showing organdy covered pots

3.3 Results and Discussion

In all of the test entries, aphid numbers were highest (concentrated) on the upper part of the stem during the screening period. This may be due to a concentration of amino acids more on the phloem than on the leaves.

Results of the experiment revealed that number of aphids on each cultivar varied widely throughout the screening ($F = 6.20$; $df = 49,98$; $P < 0.001$) proving existence of genetic variability for resistance against South African pea aphid strain (Table 3.1). Differences in the damage caused by an insect to different plants in a locality during a given period reflect their relative susceptibility or resistance.

Most entries (32 out of 50) were moderately susceptible to the South African pea aphid strain (Fig. 3.2). Nine entries appeared to be susceptible while seven and one entries were moderately resistant and resistant, respectively.

Most entries from Ethiopia had susceptible reaction (Fig. 3.3). Only one entry ILL 204 was moderately resistant, harboring a mean of 49 aphids per plant. ILL 203, ILL 509 and ILL 1917were moderately susceptible while ILL 205, ILL 206, ILL 207, ILL 247, ILL 208, and ILL 626 were susceptible, harboring mean population exceeding 100 aphids per plant. The majority of Syrian (ICARDA) advanced lines were susceptible to moderately susceptible to *A. pisum*.

Almost all advanced lines (14) from USA were moderately susceptible to the South African *A. pisum* population. Brewer and LC 660615L were moderately resistant. Among cultivars from Lesotho, three were susceptible while French Indigo was moderately resistant. There were dead aphids on this cultivar, which might have been due to the presence of antibiotic component in the plant. Two Canadian lines appeared to be moderately resistant

and one was resistant. The Spanish line, Verdina, had the lowest mean aphid population (Table 3.1). In comparison with cultivars /lines of other origin, Spanish cultivars performed well.

In general, relying on aphid numbers per a plant as a sole norm has its own shortcomings. Potentially tolerant plants with high aphid numbers might be excluded. Hence, further evaluation of susceptible plants might be necessary in order to identify potentially tolerant plants from susceptible ones.

Table 3.1 Test entries, plant introduction (PI) numbers, origin of test entries and resistance ratings of lentil entries included in the tests.

Test entry	PI number	Origin	Number of aphids	Resistance rating
ILL 8127	?	ICARDA/Syria	14 (196)	S
ILL 7981	?	ICARDA/Syria	13 (169)	S
ILL 626	PI 358602	Ethiopia	13 (169)	S
ILL 208	PI 193817	Ethiopia	13 (169)	S
ILL 207	PI 193550	Ethiopia	13 (169)	S
ILL 247	PI 273664	Ethiopia	13 (169)	S
ILL 206	PI 193549	Ethiopia	12 (144)	S
ILL 6024	?	ICARDA/Syria	12 (144)	S
ILL 205	PI 193548	Ethiopia	11 (121)	S
Laird Lentil	PI 607915	Canada	10 (100)	MS
ILL 203	PI 193546	Ethiopia	10 (100)	MS
Berati	PI 606693	USA	10 (100)	MS
ILL 5883	?	ICARDA/Syria	10 (100)	MS
ILL 7012	?	ICARDA/Syria	10 (100)	MS
Adi		Turkey	10 (100)	MS
WA 8649085	PI 547038	USA	10 (100)	MS
LC 460212L	-	USA	10 (100)	MS
Benewah	PI 564719	USA	10 (100)	MS
Mokhotlong Local	?	Lesotho	10 (100)	MS
Chilean 78	PI 477920	USA	10 (100)	MS
LC 7601606T	-	USA	10 (100)	MS
ILL 7005	?	ICARDA/Syria	9 (81)	MS
ILL 7620	?	ICARDA/Syria	9 (81)	MS
ILL 6994	?	ICARDA/Syria	9 (81)	MS
Mason	?	USA	9 (81)	MS
Indian Head	PI 606659	Canada	9 (81)	MS
ILL 4400	?	ICARDA/Syria	9 (81)	MS

Table 3.1Continued...

Test entry	PI number	Origin	Number of aphids	Resistance rating
Raja	?	Lesotho	8 (64)	MS
Palouse	PI 557499	USA	9 (81)	MS
Redchief	PI 477921	USA	9 (81)	MS
ILL 1917	PI 193545	Ethiopia	9 (81)	MS
ILL 509	PI 320941	Ethiopia	9,(81)	MS
ILL 7696	?	ICARDA/Syria	8 (64)	MS
Macro	?	Lesotho	8 (64)	MS
LC 460197L	-	USA	8 (64)	MS
LC 660087L	-	USA	8 (64)	MS
LC 560266L	-	USA	8 (64)	MS
ILL 8090	?	ICARDA/Syria	8 (64)	MS
Easton	PI 471917	Canada	8 (64)	MS
LC 7601682T	-	USA	8 (64)	MS
LC 760960E	-	USA	8 (64)	MS
LC 660272L	-	USA	8 (64)	MS
WA 8649041	PI 547039	USA	7 (49)	MR
Brewer	PI 508090	USA	7 (49)	MR
Pardina	PI 533690	Spain	7 (49)	MR
ILL 204	PI 193547	Ethiopia	7 (49)	MR
Spanish Brown	PI 565081	Spain	6 (36)	MR
French Indigo	?	Lesotho	6 (36)	MR
LC 660615 L	?	USA	5 (25)	MR
Verdina	PI 533693	Spain	4 (16)	R

CV (%)= 16.8

LSD value (P< 0.05) = 32.36

? = Unknown

The square root of the total aphid numbers is given with the back transformed data in brackets

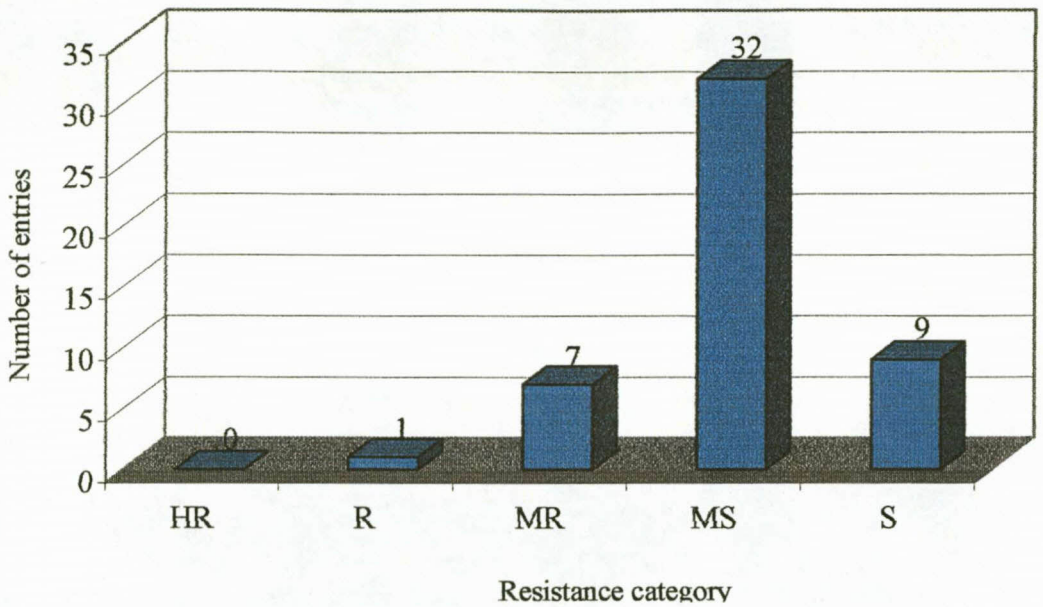
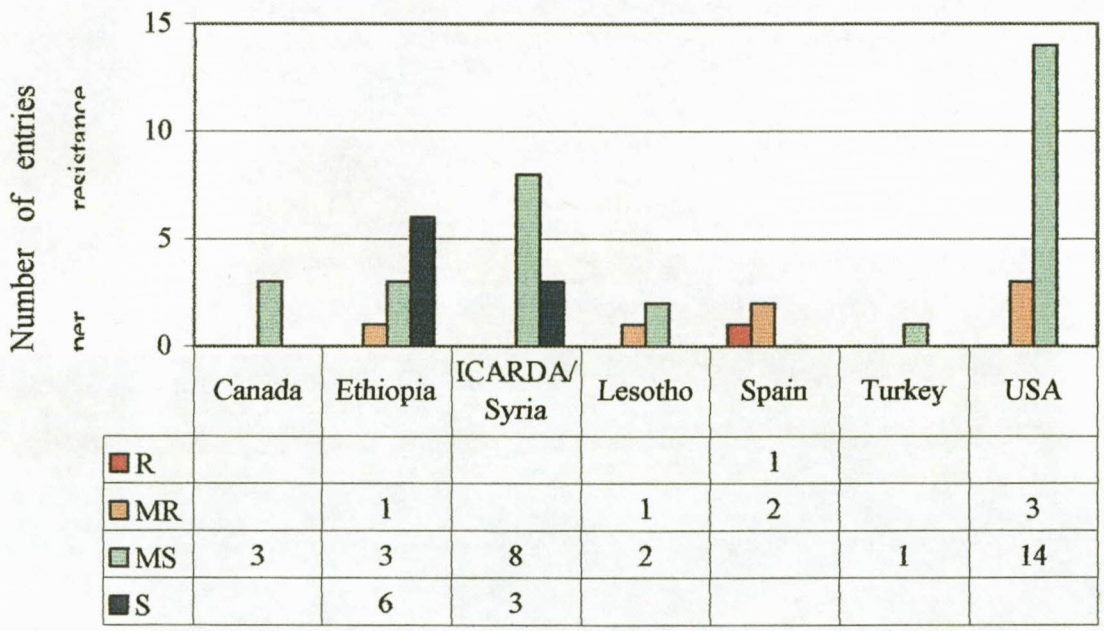


Fig.1. Frequency distribution of resistance ratings of fifty entries of lentil evaluated against *A. pisum*



	Canada	Ethiopia	ICARDA/ Syria	Lesotho	Spain	Turkey	USA
R					1		
MR		1		1	2		3
MS	3	3	8	2		1	14
S		6	3				

Fig. 2 Resistance category of test entries per country of origin

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CHAPTER 4

Mechanisms of resistance to *Acyrthosiphon pisum* (Harris) (Homoptera: Aphididae) in lentil entries

Abstract

Glasshouse experiments were conducted to determine categories of resistance of five entries of lentil, *Lens culinaris* Medikus (French Indigo, Spanish Brown, Verdina, Brewer and LC660615L) previously identified as resistant to *Acyrthosiphon pisum*. One aphid-susceptible line 'ILL 8127" was also included for comparison. Results of the antibiosis experiment revealed that French Indigo displayed high antibiotic reaction (increased mortality of adults, decreased fecundity, post-reproductive period and longevity). Total mean fecundity per adult *A. pisum* on French Indigo, Spanish Brown, Verdina and LC660615L was 9, 37.1, 42.1 and 33.3, respectively, compared with 80.1 nymphs per adult on ILL 8127. Analysis of antixenotic experiment data indicated that French Indigo, Spanish Brown, Verdina and Brewer appeared to be anixenotic while the tolerance component indicated Spanish Brown as the only tolerant line. Overall, Spanish Brown appeared to be promising having antibiosis, antixenosis and tolerance to *A. pisum*.

Key words: Lentil, *A. pisum*, mechanisms of host plant resistance

4.1 Introduction

Plants and insects have a long time co-existed relationship. Plants may have suppressed harmful pests through their natural chemicals that adversely affect insects feeding on them or with their hairy leaves and thick waxy cuticles that deter insect feeding or through tolerance to insect feeding, a mechanism that allows plants to sustain defoliation without suffering from the infestation. This natural balance between the insect pest population and hosts led entomologists focus on host plant resistance studies and harness the mechanisms.

The three classic modalities of resistance established by Painter (1951) are antibiosis, tolerance and non-preference later replaced as antixenosis by Kogan & Ortman (1978). According to these authors, the three mechanisms of resistance are interrelated although they may function alone.

Antibiosis is the mechanism by which a colonized plant is resistant because it has an adverse effect on an insect's development, reproduction and survival (Dent, 2000). Kogan (1975) reported that insects fed on resistant plants may manifest antibiotic symptoms, which range from lethal or acute to very mild or sub chronic. He further stated the physiological explanations for these symptoms as:

1. Presence of toxic metabolites (alkaloids, glucosides and quinones),
2. Absence or sub optimal amounts of some essential nutrient,
3. Unbalanced proportions of nutrients,
4. Presence of antimetabolites that render some essential nutrients unavailable to insects, and;
5. Presence of enzymes that inhibit normal processes of digestion of food and consequently utilization of nutrients.

Antixenosis is the resistance mechanism employed by the plant to deter colonization by an insect (Dent, 2000). This component of resistance can be caused by physical or chemical plant factors that repel insect herbivores from feeding or oviposition (Smith, 1989).

Dent (2000) defined plant tolerance as the extent to which a plant can support an insect infestation without loss of vigor or crop yield. He stated that these mechanisms influence the population dynamics of a pest insect by their action on the life history parameters.

A. pisum is known as a pest of many legumes capable of sucking the lifeblood out of the phloem. Current strategies for the control of aphid pests in Ethiopia almost entirely depend on the application of systemic insecticides. To avoid development of resistant strains and environmental hazards caused by frequent application of insecticides, studies should focus on alternative control strategies.

Host plant resistance to *A. pisum* has been a valuable control method in different parts of the world. The development of effective and rational management of *A. pisum* however, relies on a thorough understanding of the biology of the pest (Morgan *et al.*, 2001). Despite these, studies on the modalities of resistance of lentil cultivars to *A. pisum* are at their infant stages in Ethiopia while the loss due to this pest is dramatically high every year. To combat such problem, this study addressed three hypotheses:

1. Test entries have the same amount of antibiotic resistance on different stages of *A. pisum* life cycle
2. Populations of *A. pisum* have the same preference for test entries and,
3. Test entries exposed to uniform number of *A. pisum* population are potentially tolerant.

4.2 Materials and Methods

4.2.1 Aphid colony

Technique for rearing aphids was as described in Chapter 3. However, in this case aphids were collected from Lucerne fields at Clarens, Eastern Free State, South Africa.

4.2.2 Antibiosis

4.2.2.1 Nymph Counts

A life history analysis test was conducted to assess plant effects on various stages of the aphid life cycle. Entries selected based on the preliminary screening were planted (3 seedlings per pot) in 1 liter plastic pots filled with greenhouse soil that contained a standard soil-compost mixture (1:1 soil-peat v/v). Plants were thinned to single seedlings after emergence. The line 'ILL 8127' was included as a susceptible control. Individual two weeks old seedlings were infested with single apterous adult *A. pisum* from the stock culture using a moistened camel's-hair brush. Aphids were confined to individual plants with nylon organdy cloth to avoid aphid escape. The aphids were then allowed to reproduce for 24hrs. When reproduction commenced, adult aphids and all progeny were removed leaving only one newborn *A. pisum* on each plant. Number of molts, and progeny produced by each aphid were recorded. The progeny were removed daily till reproduction ceased.

Experimental design and analysis: The experiment was designed as randomized complete block design (RCBD) with seven replications. Life table parameters computed from the raw data included time to adulthood, time to start reproduction, mean daily nymphal production, maximum daily nymphal production, total fecundity, post-reproductive period, and life span. Other parameters calculated from the raw data included, (1) Intrinsic rate of increase (r_m) estimated as $r_m = 0.74 (\ln M_t)/t$ where M_t is number of progeny produced by

each aphid in a span equivalent to its developmental time (t) (Wyatt & White, 1977), (2) finite rates of increase (the number of individuals added to the population per female per day, or the population capacity to multiply a number of times per female per day) using the formula λ = antilog r_m. (De Loach, 1974), (3) generation time (Td) using Td = 4d/3 (Wyatt & White, 1977), where d is the time from birth to onset of reproduction and; (4) time for population to double its size (DT) estimated as DT = [$\log_e$ (2)]/rm. (De Loach, 1974).

Data on life history parameters of *A. pisum* on the six lentil entries were analyzed using a one-way analysis of variance. Because the adult female on French Indigo died on most replications, data from this cultivar was not included in analysis of r_m, λ, daily nymphal production, maximum daily nymphal production and doubling time. The least significant difference (LSD) was used to test for differences between pairs of means at $\alpha = 0.05$ (MSTATC, 1990).

4.2.2.2 Colony Counts

The procedure described in 4.2.2.1 was followed during planting and infestation. However, newborn nymphs were left on each plant and were counted when the original female in the nymph count technique died. Hence, the colony count technique included all newborn nymphs. The experiment was replicated five times in a randomized complete block design.

Data was analyzed as a randomized complete block design and means were compared using least significant difference at $\alpha = 0.05$ (MSTATC, 1990).

4.3.3 Antixenosis

This test was designed to evaluate the non-preference or antixenosis (Kogan & Ortman, 1978) component of each entry. The entries were randomized and planted in standard greenhouse soil in equidistant hills in a circular pattern near the edge of 18-cm-diameter plastic pots (Fig. 4.2). There were six hills (1 plant per hill), with one hill of each entry per pot.

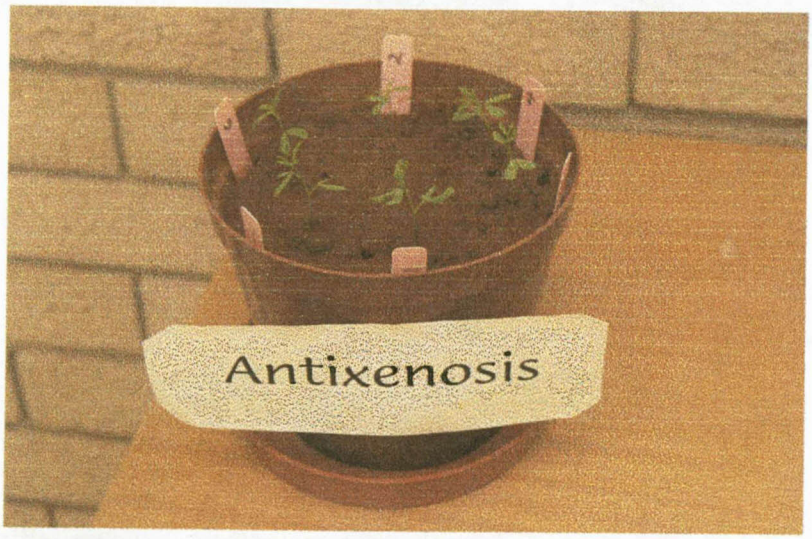

Fig. 4.2 Test plants randomized and planted equidistantly from the center.

When the plants reached 3 - 4 leaf stages, 60 aphids from the stock colony were released on the soil in the center of each pot so that all entries had an equal chance of being infested by the aphids. The number of aphids was determined to 10 aphids per plant basis according Kindler *et al.* (1995); Unger & Quisenberry (1997); Hesler *et al.* (1999) on other aphids. Cages were then covered with ventilated black muslin cloth (Fig. 4.3) to avoid natural light. Webster & Inayatullah (1988) reported that aphid migration responses might be

positively phototaxic. Hence this experiment excluded the effects of photo taxis on *A. pisum* behaviour.

Fig. 4.3 Pots covered with black muslin cloth after aphids were introduced.

Experimental pots were watered every other day and the aphids were allowed 48hrs to select the plant of their own choice, at which time the number of aphids on each cultivar was recorded and expressed as a proportion of the total *A. pisum* population introduced. Newly oviposited nymphs were not counted because they were not involved in the host selection process. The condition of the greenhouse was maintained at 20 ± 2^0C. Seven replications (pots) were included in the test as a randomized complete block.

Data analysis: collected data was analyzed by one-way ANOVA and means were separated with least significant difference at $\alpha = 0.05$ (MSTATC, 1990).

4.3.4 Tolerance

This test was conducted to measure the ability of lentil cultivars to withstand aphid infestation. Single plants of each entry were grown in 1litre plastic pots filled with standard greenhouse soil and arranged in randomized complete block design with seven replications where uninfested control plants for each test entry was also included. When the plants reach 3 - 4 leaf stage, each of the seedlings was infested with ten fourth - instar apterous pea aphids taken from the stock culture and the other were used as controls. Control plants were left uninfested and were placed beside corresponding infested plants of the same entry and replication. Both sets of plants were covered with ventilated nylon organdy cloth (Fig. 4.4) supported by aluminum steel stand, which fited to the pot to avoid aphid escape. Infested plants were examined at 48-hr intervals so that new progenies could be removed or adult aphids could be added as needed to insure uniformity in infestations to 10 adults per plant (Webster, 1990). Temperature and photoperiod in the greenhouse was maintained at 20 ± 2^0C and 16: 8 (L: D) hr respectively. Twelve days after infestation plant heights were determined by measuring the plant from base to the tip of the longest leaf blade. Number of leaves per each plant was counted. Each plant was then cut at soil level, and soil was washed from the roots. Fresh plant and root mass were taken and both were dried at 50^0C for 48hrs and weighed 3hrs after removing from oven (Unger & Quisenberry, 1997). To provide relative degree of height and weight reduction, plant heights and weights of each entry per replication were standardized according to Unger & Quisenberry (1997). i.e. infested plant values were divided by uninfested plant values (e.g., percentage of relative reduction in plant height = (1 – [infested/uninfested]) x 100.

Data analysis: all data were then analyzed as one factor randomized complete block design and means were separated using Tukey's significant difference at $\alpha = 0.05$ (MSTATC, 1990).

Fig. 4.4 Test entries during the tolerance test

4.3 Results and Discussion

4.3.1 Antibiosis

4.3.1.1 Nymph Counts

The developmental times of *A. pisum* from newborn nymph to adulthood reared on six lentil entries did not vary significantly ($F = 2.39$; $df = 5, 30$; $P > 0.05$).

Entries had a significant effect on the pre-reproductive period of *A. pisum*. The shortest pre-reproductive period (Table 1) was noted on Verdina and LC660615L but not significantly different from Spanish Brown. The pre-reproductive period on LC660615L and

ILL 8127 did not differ significantly. This period also did not differ among French Indigo, Spanish Brown and Brewer.

The influence of entries on the reproductive period of pea aphid was highly significant ($F = 35.90$; $df = 5, 30$; $P < 0.001$) (Table 4.1). The mean reproductive period *A. pisum* on French Indigo was significantly shorter than on the other entries. The reproductive period on ILL 8127 was also longer than on Verdina, Spanish Brown and LC660615L.

Table 4.1 Life table parameters of the pea aphid, *Acyrthosiphon pisum* (Harris) reared on six entries in glasshouse

Entry	Pre-reproductive period (days)	Reproductive period (days)	Post-reproductive period (days)	Longevity (days)
ILL 8127	2.00 ± 0.93bc	18.00 ± 1.51a	6.71 ± 1.48a	33.71 ± 2.12a
Brewer	3.14 ± 1.36a	17.71 ± 2.19a	5.43 ± 2.19a	32.86 ± 2.64a
Verdina	1.43 ± 0.49c	14.86 ± 1.55b	6.29 ± 1.69a	30.29 ± 2.31ab
Spanish Brown	2.43 ± 0.90abc	14.00 ± 1.51bc	5.14 ± 1.00a	29.00 ± 2.67b
LC660615L	1.71 ± 0.88c	12.00 ± 1.77c	6.14 ± 2.75a	27.29 ± 4.16b
French Indigo	2.86 ± 0.83ab	3.71 ± 3.57d	1.29 ± 1.48b	16.14 ± 4.67c

Means in each column followed by the same letter are not significantly different ($\alpha = 0.05$, LSD Test [MSTAT-C]).

Entries had a highly significant effect on pea aphid longevity ($F = 25.61$; $df = 5, 30$; $P < 0.001$). Mean longevity ranged from 33.71-16.14 days. ILL 8127 allowed *A. pisum* to live for 33.71 days although longevity was not statistically different from Brewer and Verdina. Differences in longevity were not significant ($P < 0.05$) among Verdina, Spanish Brown and LC660615L. The shortest life span was pronounced on French Indigo, which allowed *A. pisum* a mean life span of 16.14 days. This cultivar allowed the lowest fecundity, reproductive period and post-reproductive periods of *A. pisum* compared to the other entries.

Entries had a highly significant effect on daily nymphal production ($F = 15.62$; $df = 4, 24$; $P < 0.001$). Nymphs produced on all entries were significantly fewer than ILL 8127 (Fig. 4.5). Markkula & Roukka (1970) reported that *A. pisum* has higher reproductive rates on susceptible plants than on resistant hosts. Among the resistant entries, Brewer had the highest production of nymphs, although it was not significantly from Verdina and LC660615L. These figures were lower than those reported by Morgan *et al.* (2001) on pea cultivar 'Sancho'. However, such differences are due to the combined effects of different cultivars of host plants and aphid biotypes (Frazer, 1972).

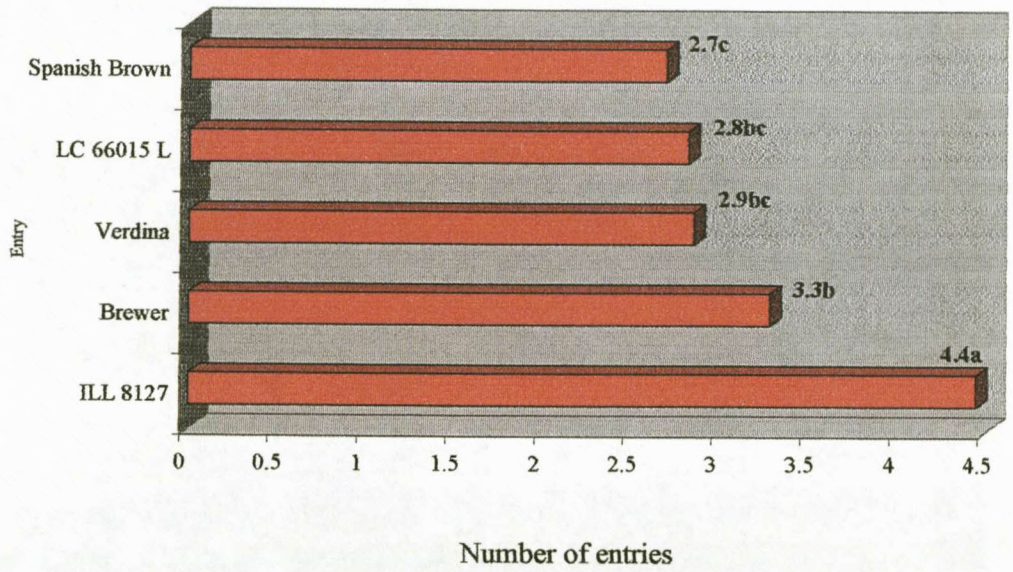

Fig. 4.5 Average number of nymphs of *Acyrthosiphon pisum* produced per day during the nymphipositional period on five lentil entries

The influence of entries on maximum daily nymphal production was significant (F = 4.64; df = 4, 24; P = 0.01). ILL 8127 allowed the maximum production of nymphs per day than other entries (Fig. 4.6). Nymphs produced on Brewer were lower although not significantly different from ILL 8127. The influence of Verdina, Spanish Brown and LC660615L on maximum number of progenies produced in a single day was not statistically different.

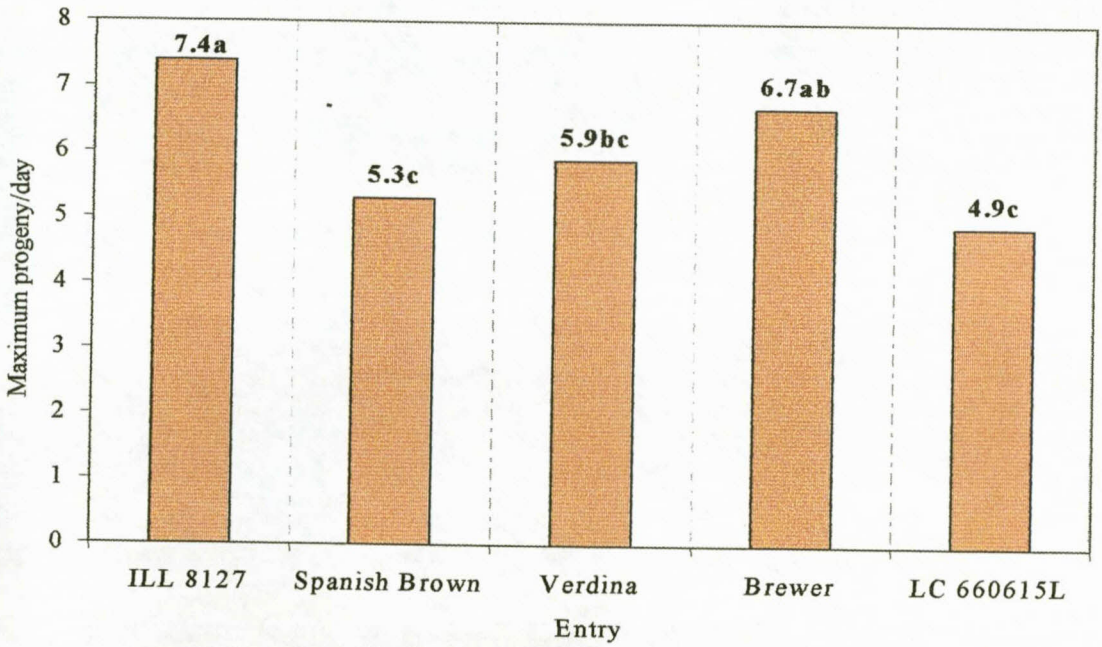

Fig. 4.6 Maximum progeny of *Acyrthosiphon pisum* per female per day during the nymphipositional period

Highly significant differences in the reproductive capability of *A. pisum* on six entries were recorded (F = 57.60; df = 5, 30; P < 0.001). Mean fecundity was higher on ILL 8127 and least on French Indigo (Fig. 4.7). Among the resistant cultivars, Brewer had the highest number of aphids. LC660615L, Spanish Brown and Verdina were not significantly different from each other.

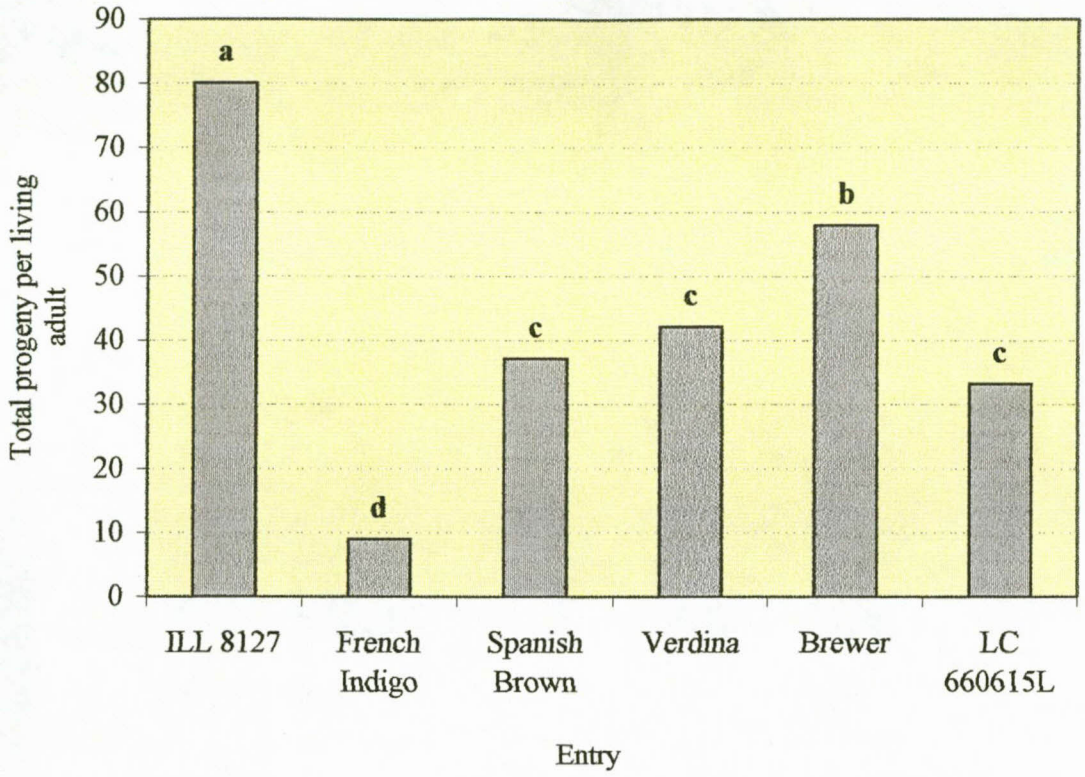

Fig. 4.7 Total nymphs per female of *A. pisum* on six lentil entries

According to Kennedy & Stroyan (1959), fecundity varies between individuals with in the same species. Several workers (Van Emden, 1972; Mansour *et al.*, 1982; Leather *et al.*, 1985; Soroka & Mackay, 1991) mentioned that host plant effects are one of the biotic factors, which influence aphid fecundity. In this study, adult oviposition rates fed on French Indigo were greatly reduced (Fig. 4.7). Aphids feeding on antibiotic plants can experience reduced body size, delayed development, decreased longevity and reduced fecundity (Panada, 1979). Kindler *et al.* (1995) explained decreased fecundity in aphids as the detrimental effects of antibiosis on pest biology. In more detailed, Rafi *et al.* (1996) reported it as an adverse interaction between the allelochemicals in resistant genotype tissue and aphids. Therefore, the lowest adult oviposition and continued succession of nymph mortality evidenced on French Indigo indicates the influence of antibiotic factor (s) in this cultivar. In this study, all

parameters taken to assess antibiotic plant resistance showed that the level of antibiosis in LC660615L, Spanish Brown and Verdina is higher than the susceptible line. However, as suggested for other aphids' (Unger & Quisenberry, 1997) further examination of the presence or absence of constituents in 'antibiotic' plants that affect insect development and survival is needed to assess the role of antibiotic lentil plant resistance in *A. pisum* population.

The intrinsic rate of increase is a basic parameter, which an ecologist may wish to establish for an insect population. It is defined as the rate of increase per head under specified physical conditions, in an unlimited environment where the effects of increasing density do not need to be considered (Birch, 1948). Significant differences in intrinsic rate of increase of *A. pisum* were observed among entries ($F = 5.19$; $df = 4,24$; $P = 0.0034$). The r_m values were higher on ILL 8127, the susceptible control than on the other entries. Higher instantaneous rates of increase of *D. noxia* have also been reported on susceptible line of wheat than resistant lines (Formusoh *et al.*, 1994). Kaakeh & Dutcher (1993) relate higher r_m values to differences in patterns of fecundity. However, these differences in r_m values may also be explained by the influence of different pre-reproductive periods.

The lowest r_m for *A. pisum* was recorded on Spanish Brown although the r_m on this line was not significantly different from LC660615L and Verdina. The r_m values reported here are within the ranges reported for *A. pisum* on peas, beans, alfalfa and lentil (Frazer, 1972; Siddiqui & Barlow, 1973; Mackay & Wellington, 1975; Campbell & Mackuer, 1977; Kaakeh & Dutcher, 1993; Damte, 1999; Morgan *et al.*, 2001) and for other aphids (De Loach, 1974). In general, the results demonstrate that ILL 8127 would most likely harbor high *A. pisum* populations than other entries.

Differences among entries in finite rates of increase (λ) are indicated in Table 4.2. On ILL 8127 with the maximum r_m, *A. pisum* had the potential to multiply by 1.422 times per day. The lowest rates of increase were noted on Spanish Brown.

Differences in mean generation times of *A. pisum* were significant among some entries ($F = 4.16$; $df = 5, 30$; $P = 0.01$). On ILL 8127, A. pisum had a generation time in between those indicated for the other resistant entries. The longest generation time of *A. pisum* was recorded on French Indigo while the shortest was on Verdina.

Entries had a significant effect on days for *A. pisum* populations to double. *A. pisum* required longer time to double its population on Spanish Brown and LC660615L as compared to ILL 8127, the susceptible control. This indicates that *A. pisum* had the capacity to double every 1.99 days on ILL 8127 than on other entries. However, ILL 8127 did not vary significantly from Brewer and Verdina. Doubling times on LC660615L, Verdina and Brewer were not statistically different from each other ($F = 4.90$; $df = 4,24$; $P = 0.005$).

Table 4.2 Demographic statistics of pea aphid reared on six lentil entries

Test entries	**Intrinsic rate of increase, r_m**	**Finite rate of increase, λ**	**Generation time (days)**	**Doubling time (days)**
ILL 8127	0.352 ± 0.04a	1.422	3.048	1.993
Brewer	0.327 ± 0.04ab	1.387	3.619	2.159
Verdina	0.310 ± 0.05abc	1.363	1.905	2.297
Spanish Brown	0.267 ± 0.03c	1.306	3.619	2.638
LC660615L	0.280 ± 0.02bc	1.323	2.476	2.491
French Indigo	NI	NI	3.810	NI

Means in each column followed by the same letter are not significantly different ($\alpha = 0.05$, LSD Test [MSTAT-C]).
NI = Not included in the analysis

Life history parameters of *A. pisum* discussed so far indicate clear differences between entries. French Indigo appeared to be highly antibiotic. Aphids on this cultivar had all the symptoms of antibiotic effects of plant resistance. Spanish Brown and LC660615L were also antibiotic as aphids reared on them had a short reproductive period, low fecundity and a short lifespan. Verdina has also some antibiotic plant resistance. In contrast to these entries, Brewer showed no antibiotic plant resistance, because the reproductive period, post-reproductive period, maximum daily nymphal production, longevity and r_m did not differ from ILL 8127, the susceptible control.

4.3.1.2 Colony Counts

Number of aphids on the six entries of lentil varied widely ($F = 21.75$; $df = 4, 16$; $P < 0.001$). Aphid colonies were higher on ILL 8127 as compared with the resistant entries (Table 4.3). All resistant plants had substantial amount of antibiosis host resistance relative to ILL 8127, the susceptible control. Aphids on French Indigo data not included in the analysis, died on most replications. This is most likely due to the antibiotic properties exhibited by the cultivar.

In general, removing nymphs on daily basis (nymph count technique) to assess antibiotic plant resistance was the reliable technique as it attributed a specific cause of antibiosis with in each entry. Scott *et al.* (1990) reported that this technique is designed to discriminate among varieties for their ability to reduce fecundity of aphids. On the contrary, colony count technique allowed antibiotic expression in more generalized way i.e. fewer fecundity and shorter longevity of *A. pisum.* This technique may be more realistic indictor of antibiosis (Scott *et al.*, 1990), for agronomists and breeders, than nymph counts since their interest is in the broad modes of resistance.

Table 4.3 Number of progenies of *A. pisum* on colony counts technique

Test entry	Number of progeny
ILL 8127	105.4a
Brewer	60.80b
LC660615L	57.40b
Verdina	38.60c
Spanish Brown	34.6c

Means in each column followed by the same letter are not significantly different ($\alpha = 0.05$, LSD Test [MSTAT-C]).

4.3.2 Antixenosis

Highly significant differences in preference of *A. pisum* were noted among entries ($F = 51.25$; $df = 45$; $P < 0.001$). Kogan (1975) reported that phytophagous insects usually display a consistent pattern of preferences when offered a choice of two or more alternative foods. ILL 8127 was the most preferred and Spanish Brown the least preferred (Table 4.4). Preference for Brewer, LC660615L and ILL 8127 also differed ($F = 51.25$; $df = 5, 45$; $P < 0.0001$). LC660615L harbored many aphids, which indicated a relatively less antixenotic resistance. No significant difference was noted between Verdina and French Indigo.

All entries showed different levels of antixenotic resistance as compared to ILL 8127. Aphids concentrated more on ILL 8127 than other entries. In their study, Kishaba & Manglitz (1965) reported that aphids aggregated on susceptible clones of alfalfa and clover than on the resistant plants after 20 hours. Dent (2000) explained in such a way that plants with antixenotic resistance would be expected to have reduced initial infestation than susceptible plants. The study showed that pea aphids selected (colonized) cultivars with relatively low percentage of antixenotic resistance given multi-choice conditions.

Table 4.4 Preference of *Acyrthosiphon pisum* for six lentil entries measured as the percent of aphids on each entry

Entry	**PI number**	**Origin**	**%Pea aphids/plant ± SEM after 48hr**
ILL 8127(Control)	?	ICARDA/Syria	29.2 ± 4.2a
LC660615L	?	USA	19.8 ± 2.3b
Brewer	PI 508090	USA	16.7 ± 3.3c
Verdina	PI 533693	Spain	11.8 ± 2.4d
French Indigo	?	Lesotho	9.8 ± 1.6de
Spanish Brown	PI 565081	Spain	8.2 ± 3.6e

Means in each column followed by the same letter are not significantly different ($P \leq 0.05$, LSD test [MSTAT-C]).

Means of 10 replications.

4.3.3 Tolerance

Highly significant differences in plant height were established among entries in both infested and uninfested plants at $\alpha = 0.05$. Infested seedlings had significantly lower ($F = 6.44$; df = 5, 30: $P = 0.0004$) plant height than uninfested seedlings (Table 4.5). Among infested entries, no significant difference was noted between ILL 8127 and the resistant entries. However, various workers (Bush *et al.*, 1989; Du Toit, 1989; Scott *et al.*, 1991) reported that severe plant height reduction can occur in a resistant line despite high level of resistance in damage rating.

Percentage plant height reduction was also significantly different between entries ($F = 3.87$; df = 5, 30; $P = 0.01$). The percentage plant height reduction of resistant entries differed

from that of ILL 8127 (Table 4.5). Spanish Brown displayed the lowest reduction in plant height.

Table 4.5 Plant height of infested and uninfested entries and percent reduction in plant height

	Plant height (cm)		
Test entry	**Infested[a]**	**Uninfested[b]**	**Percent reduction[c]**
ILL 8127	6.8ab	15.1a	55.48a
Brewer	7.1a	14.9a	50.45b
LC660615L	5.7b	10.9b	46.72c
Verdina	5.7b	10.6b	44.48c
French Indigo	5.7b	10.3b	44.33c
Spanish Brown	7.6a	11.0b	31.10d

[a] SEM = 0.35; LSD = 0.33

[b] SEM = 0.59; LSD = 0.54

[c] SEM = 4.50; LSD = 0.74

Means in each column followed by the same letter are not significantly different (P = 0.05, Tukey's Significant Difference Test [MSTAT-C]).

Means of 7 replications.

The number of leaves of infested and uninfested entries of lentil is shown in Table 4.6. There were significant differences in the number of leaves on both infested and uninfested plants. All of the infested seedlings had significantly lower ($F = 3.48$; $df = 5, 30$; $P = 0.01$) leaves than uninfested seedlings ($F = 8.12$; $df = 5, 30$; $P = 0.0001$). Uninfested seedlings of all entries had at least twice the number of leaves than infested seedlings. Except for Spanish Brown, uninfested resistant entries were not significantly different from ILL 8127. The number of leaves on infested ILL 8127 seedlings did not differ from that on the resistant entries. However, the number of leaves on Spanish Brown differed from the number on Verdina and French Indigo.

Table 4.6 Number of leaves of infested and uninfested plants of lentil at the end of the experiment

	Number of leaves	
Test entry	**Infested[a]**	**Un-infested[b]**
ILL 8127	13.57ab	35.43b
Brewer	13.00ab	35.00b
LC660615L	13.71ab	33.71b
Verdina	9.71b	33.71b
French Indigo	9.57b	32.14b
Spanish Brown	15.43a	41.86a

[a] SEM = 1.36; LSD = 1.26

[b] SEM = 1.29; LSD = 1.20

Means in each column followed by the same letter are not significantly different (P = 0.05, Tukey's Significant Difference Test [MSTAT-C]).

Means of 7 replications.

Infested seedlings differed in their fresh plant weight. Fresh plant weight was low for French Indigo, which was the only cultivar statistically different from ILL 8127 ($F = 3.88$; $df = 5, 30$; $P = 0.01$).

Differences in fresh plant weight among uninfested entries were highly significant ($F = 32.85$; $df = 5, 30$; $P < 0.001$). Brewer showed more fresh plant weight (0.36gm) than all test entries (Table 4.7). ILL 8127, LC660615L and Spanish Brown were intermediate to Brewer, not differing significantly from each other. The fresh plant weight of Verdina was low but not different ($P < 0.05$) from French Indigo. Despite the differences noted on infested and uninfested entries, percentage reduction in fresh plant weight was not significant ($F = 1.91$; $df = 5, 30$; $P = 0.12$).

Table 4.7 Fresh plant weight (gram) of infested and uninfested entries of lentil after twelve days

	Fresh plant weight (g)	
Test entry	***Infested***[a]	***Un-infested***[b]
ILL 8127	0.11a	0.26b
Brewer	0.09ab	0.36a
LC660615L	0.09ab	0.21bc
Verdina	0.07ab	0.14d
Spanish Brown	0.09ab	0.25b
French Indigo	0.06b	0.16cd

[a] SEM = 0.01; LSD = 0.011

[b] SEM = 0.01; LSD = 0.012

Means in each column followed by the same letter are not significantly different (P = 0.05, Tukey's Significant Difference Test [MSTAT-C]).

Means of 7 replications.

Aphid infestation had a significant effect on dry plant weight of the six entries (F = 4.58; df = 5, 30; P = 0.003). All the resistant lentil entries were not significantly different from ILL 8127 (Table 4.8). But among the resistant entries, Verdina and French Indigo had the lowest dry plant mass and were significantly different from Spanish Brown at 5% significant level. On the other hand, uninfested seedlings also varied in their dry plant weight (F = 10.82: df = 5, 30; P < 0.0001). Brewer had the highest dry plant weight and was different from all test entries including ILL 8127. Other entries did not vary in dry plant weight at 5 % significant level. Although differences in infested and uninfested seedlings were noted, percentage dry plant weight reduction was not statistically significant (F = 2.23; df = 5, 30; P = 0.07).

Table 4.8 Dry plant weight (gram) of infested and uninfested entries of lentil at the end of the experiment

	Dry plant weight (g)	
Test entry	***Infested***[a]	***Un-infested***[b]
ILL 8127	0.02ab	0.04b
Brewer	0.02a	0.06a
Spanish Brown	0.02ab	0.05b
LC660615L	0.02ab	0.03b
Verdina	0.01b	0.03b
French Indigo	0.01b	0.03b

[a] SEM = 0.002; LSD = 0.002

[b] SEM = 0.004; LSD = 0.04

Means in each column followed by the same letter are not significantly different (P = 0.05, Tukey's Significant Difference Test [MSTAT-C]).

Means of 7 replications.

Analysis of fresh root weight data indicated that there was no significant difference among infested (F = 1.31; df = 5, 30; P = 0.3) as well as uninfested entries (F = 5.48; P = 0.4) of lentil. Mean fresh root weights for infested and uninfested plants were 0.002 and 0.03 g respectively.

Dry root weights of infested and uninfested entries of lentil are indicated in Table 4.9. Differences in dry root weight data were significant (P < 0.05) among entries of both infested and uninfested seedlings. Uninfested plants had dry root weight twice (F = 5.48; df = 5, 30; P = 0.001) that of infested entries (F = 3.13; df = 5, 30; P = 0.02). All resistant entries of infested plants did not vary from ILL 8127. Brewer had more dry root weight although not significantly different from all but Verdina. Similarly in uninfested trial, most uninfested entries were not statistically (P < 0.05) different from ILL 8127 (Table 9). Brewer had

significantly the highest dry root weight. Spanish Brown was also intermediate but not significantly different from ILL 8127, LC660615L and French Indigo.

Data of dry root weight of uninfested entries was not distributed normally. Because the data appeared to be high in its coefficient of variation (53.62%) but too small for log transformation, percent reduction in dry root weight was not calculated. It is therefore concluded that dry root weight was not a good indicator of tolerance in this experiment.

Table 4.9 Dry root weight of infested and uninfested entries after twelve days

	Dry root weight (g)	
Test entry	***Infested***	***Uninfested***
ILL 8127	0.01ab	0.03bc
Brewer	0.02a	0.7a
LC660615L	0.01ab	0.3bc
French Indigo	0.01ab	0.3bc
Spanish Brown	0.01ab	0.4b
Verdina	0.01b	0.2c
SEM	*0.002*	0.01
Tukey's	*0.001*	*0.001*
P	0.02	0.001

In general, plant height was the most reliable parameter in assessing tolerance among test entries. From the percent height reduction data, it is evident that Spanish Brown possesses a significant level of tolerance to *A. pisum*. Tolerance, as a mechanism of resistance may provide resistance that is more stable than antibiosis and antixenosis (Smith, 1989).

4.3.4 Resistance Index

Normalized indices (RI) for the three components of resistance and the resistance index (RI) (Webster *et al.*, 1987; Robinson, 1992) are shown in Table 4.10. The resistance

index ranks the entries in terms of the combination of their resistance components and does not indicate any statistical difference (Robinson, 1992).

Table 4.10 Normalized indices and overall resistance index (RI) based on mechanisms of resistance to pea aphid in six lentil entries.

Entry	Antibiosis (X)[a]	Tolerance (Y)[b]	Antixenosis (Z)	RI [c]
ILL 8127	1.0	1.00	1.0	1.0
Brewer	0.72	0.91	0.57	2.68
LC660615L	0.42	0.84	0.68	4.17
Verdina	0.53	0.80	0.41	5.75
French Indigo	0.11	0.80	0.34	33.42
Spanish Brown	0.46	0.56	0.28	13.86

[a] Values based on mean nymph counts on plants

[b] Values based on percentage plant height reduction

[c] RI = 1/(XYZ)

Based on antibiosis data, French Indigo appeared to be highly antibiotic. The antixenotic component also indicated the presence of antixenotic plant resistance in this cultivar. Therefore it is possible to say that the antibiotic property expressed in it may also be due the effect of antixenotic resistance. Antixenotic plant resistance is known to deter feeding which may affect the aphid's biology. Unger & Quisenberry (1997) reported such an influence in wheat line 'PI 225245' against *D. noxia*.

Spanish Brown showed strong antixenotic, tolerance and antibiotic plant resistance. Verdina also expressed antixenotic and antibiotic components of resistance. LC660615L has more of antibiotic property. Neither antixenotic nor tolerance is found in it. Brewer appeared

to be the least of all. This entry may have some antixenotic property but no antibiotic or tolerance component of resistance was noted.

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CHAPTER 5

Evaluation of *Beauveria bassiana* on populations of *Acyrthosiphon pisum*

Abstract

The potential of three aphid-derived isolates of *Beauveria bassiana* was evaluated against *Acyrthosiphon pisum* under glasshouse condition. One aphid-susceptible line 'ILL 8127' was planted (3 seeds per pot) in 1liter plastic pots filled with standard greenhouse soil in a randomized complete block design with five replications. Seedlings were thinned to one after emergence. When the seedlings were 10 days old, twenty-five fourth instar aphids were transferred individually with a camel's hairbrush to the 10-day-old lentil seedlings. Aphids were then inoculated with the spore suspension (10^6 conidia/ml) of each the three isolates by direct spraying and pots were covered with plastic bags for 48hrs to maintain high relative humidity. Untreated plants were also included for comparison. The plastic bags were removed and pots were covered with ventilated nylon organdy to avoid aphid escape. Plants were watered every other day and temperature of the greenhouse was maintained at 22^0C for duration of the experiment. Each pot was carefully opened and inspected daily over the 10-d period. Mortality and nymph production were recorded for each aphid. New born and dead aphids were assigned an identification number and plated to determine fungal sporulation. Analysis of the data indicated that *Beauveria bassiana* significantly increased mortality of *A. pisum*. Mean mortality of aphids treated with ARSEF 2883, ARSEF 5493 and ARSEF 5705 were 94 ± 1.96, 94 ± 3.02, and 94 ± 1.96, as compared to 26 ± 4.08 for untreated aphids. Over the 10-d period, infected aphids produced significantly ($P < 0.05$) fewer aphids than untreated aphids. However, daily nymphal production was not affected by the fungal isolates. Most of the progeny were not infected with *B. bassiana* after recovery.

Key words: *Acyrthosiphon pisum*, *Beauveria bassiana*, mortality, fecundity.

5.1 Introduction

As public opposition to insecticide use increases due to human and environmental health concerns, alternative methods for controlling agricultural pests must be found in order to maintain adequately high crop yields (Armer *et al.*, 2000). In response to these problems, the discipline of integrated pest management (IPM) has evolved a fundamental principle of managing pest species based on intimate knowledge of pest population dynamics and associations with the ecosystem.

One promising avenue for research is the application of biological control in greenhouses. Insect-pathogenic fungi are considered by some as promising natural enemies for applied aphid biological control.

Aphids serve as food for a great variety of insects and are subject to infection by fungi. In spite of the premature mortality caused by biological agents, by the aphids' own activities, and by adverse physical conditions, aphids not only persist but often attain population densities that cause losses to crops. Insecticides have helped to reduce crop losses, but the chemicals are temporarily effective and the use of nonselective insecticides may actually aggravate aphid problems.

Currently, widely publicized environmental concerns and health risks associated with the use of synthetic chemical insecticides have stimulated efforts to develop biological control agents as alternatives or supplements to these chemicals (Hajek & St. Leger, 1994).

Aphids are excellent candidates for biological control (Hayden *et al.*, 1992) and a variety of fungal pathogens are available as potential control agents of aphids. Several workers (Burges, 1981; Carruthers & Soper, 1987; Mac Leod, 1963; Mc Coy *et al.*, 1988) reported that fungal pathogens are important natural biological control agents of many insects

and other arthropods and frequently cause epizootics that significantly reduce host populations. These fungi normally invade via the external cuticle and need not be ingested to initiate disease. This makes them primary candidates for use against plant sucking insects (Lacey & Goettel, 1995).

The first microorganism to be recognized as a disease agent was the fungus *Beauveria bassiana* (Bassi, 1835). It occurs worldwide and, has one of the largest host lists among the imperfect fungi and it occurs in soil as a ubiquitous saprophyte (Mc Coy *et al.*, 1988; Tanada & Kaya, 1993). However, in comparison with chemical insecticides, the adverse effects of entomopathogenic fungi to non-target organisms are generally minimal (Goettel *et al.*, 1990).

Definite limitations on their manipulation as biological control agents exist, and some researchers feel that fungus - induced epizootics are too dependent on high host densities and environmental conditions, particularly high levels of moisture (Bucher, 1964). The majority of researchers who study insect pathogens, however, believe that fungi will play a vital role in IPM systems in the near future (Allen *et al.*, 1978; Carruthers & Soper, 1987; Fuxa, & Tanada, 1987; Mc Coy *et al.*, 1988). Hence, this study addressed two hypotheses:

1. Adult *A. pisum* infected with *B. bassiana* isolates produce few progenies than uninfected aphids
2. New progenies born to infected adult *A. pisum* acquire the pathogen prior to of birth.

5.2 Materials and Methods

Aphid Colony: Rearing of aphids for this study was the same as the procedures described in previous chapters.

Origin of *Beauveria bassiana* Strains: Pure cultures of three strains of aphid-derived *B. bassiana* obtained from the Agricultural Research Service collection of Entomopathogenic fungi (ARSEF), Ithalca, NY were used (Table 5.1).

Table 5.1 *Beauveria bassiana* isolates used during the study

ARSEF	Host	Plant	Country of origin
2883	*Schizaphis graminum*	Winter wheat	USA
5493	*Aphis gossypi*	?	USA
5705	*Duraphis noxia*	*Triticum aestivum*	South Africa

? = Unknown

Production of conidia: The technique described by Vandenberg (1996) was used. Sabouraud dextrose yeast agar (SDAY) (40g dextrose, 10g peptin, 15g agar, 10g yeast extract, and 40mg tetracycline) to which 1,000ml of distilled water was added and autoclaved for 20 minutes. The solution was cooled and transferred in to sterilized plastic petri dishes (8.5cm-diameter) that were then inoculated with conidia in a laminar flow cabinet. The petri dishes were sealed with parafilm and incubated at 24^0C and a photoperiod of 15:9 (L: D) h to induce growth and sporulation of the fungus. After 10 days, the conidia were harvested by scrapping off from the surface of each petri dish (Fig. 5.1) using a sterile bacteriological loop 'police man' and were immediately stored as a slant culture at 4^0C until use.

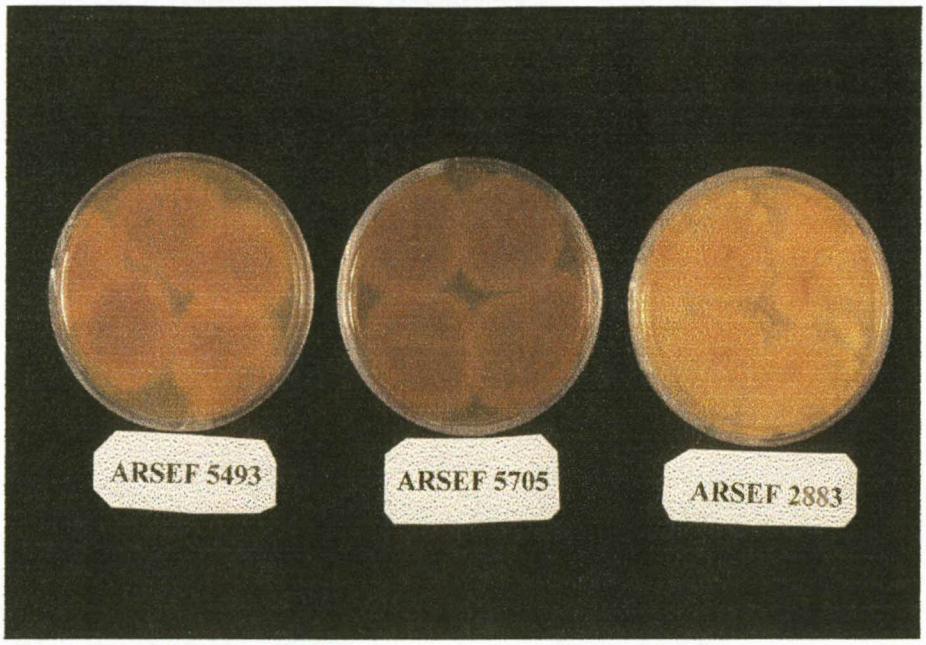

Fig. 5.1 *Beauveria bassiana* isolates on Sabraud dextrose yeast agar

Viability of conidia: The viability of conidia was verified before carrying out the study using the micro-culture method of Jimenez (1989). The microcultures were prepared by placing a layer of Sabouraud dextrose yeast agar (SDAY) on the surface of cover slips that were then inoculated with the fungus. The inoculum was prepared by suspending 0.3mg of conidia in 5ml of sterilized distilled water and homogenizing the solution with 0.025% Triton X-100 in a glass tube with a screw top in a centrifuge at high velocity (11,000 x g) for 45s. From the ensuing suspension, 0.3ml aliquots (10^6 spores per milliliter) were taken using a sterile pipette to inoculate the SDAY. Once prepared the cover slips were placed in petri dishes on damp filter paper and incubated for 36hrs at 27 ± 2^0C and 80 ± 5% RH. The micro-cultures were observed using a compound microscope, and viability was determined by the presence of 100-200 conidia per cover slip. A spore was considered viable if the germination tube was apparent. A randomized complete block with three replications was used.

Bioassay against *A. pisum*: The procedure of Vandenberg (1996) was used to grow the spores for use in the assays. Fourteen days after incubation, fungal spores from the surface of the culture were flooded with distilled water and scrapped with a sterile scalpel to make conidial suspensions. The concentration of spores was determined using a hemocytometer. It was then adjusted to 10^6 conidia per ml with sterilized distilled water (James *et al.*, 1995). A water control (sterile distilled water plus 0.02% Tween 20) was also included. Twenty-five fourth instar aphids were transferred individually with a camel's hairbrush to each of 10days old lentil seedlings. The conidiospore suspensions were then sprayed onto the plants exposing the aphids to the spores. The pots were covered with plastic bags for 48hrs to maintain high relative humidity. The plastic bags were removed and pots were then covered with ventilated nylon cheesecloth to avoid aphid escape (Fig. 5.2). Plants were watered every other day. The temperature in the greenhouse was maintained at 22^0C for duration of the experiment.

Sampling: Each pot was carefully opened and inspected daily for over the 10 days period. Nymph production as a measure of fecundity and mortality were recorded for each aphid. All newborn progeny and dead aphids were removed daily, assigned an identification number and plated on Sabraud's agar plus 1% yeast extract and kept at 27 ± 2^0C and 80 ± 5% RH. Plates were examined for 3-5 days until diagnosis based on fungal sporulation could be made. Adult mortality was attributed to mycosis only when *B. bassiana* was recovered from plated cadavers. The experiment was replicated five times in a randomized complete block design.

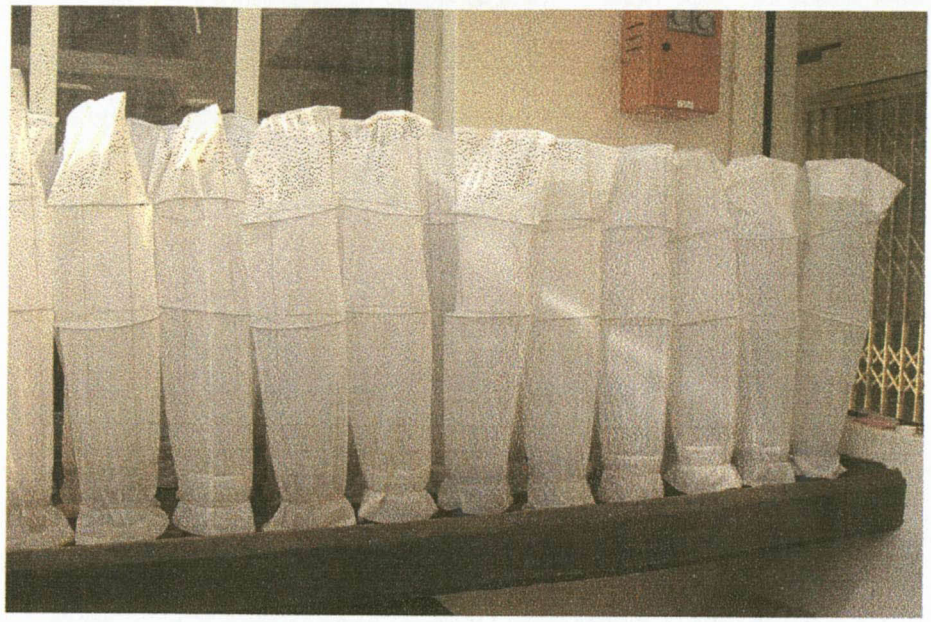

Fig. 5.2 Test plants used in bioassays of *Beauveria bassiana* against *Acyrthosiphon pisum* covered with ventilated cheese cloth

Data analysis: Cumulative mortality (proportion of the initial aphids) was calculated and converted to percentage; daily number of nymphs produced per living adult, total number of nymphs produced per adult and viability of the isolates were compared by treatment using one-way ANOVA and means were compared using LSD test at $\alpha = 0.05$ (MSTAC-C, 1990).

5.3 Results and Discussion

Viability of conidia: The viability of the conidia was high in all replications of the three *B. bassiana* isolates. There was no difference in viability between the three isolates ($F = 3.54$; $df = 2, 4$; $P = 0.13$). Percent viability of the isolates was 96.3 and or ARSEF 2883, 97.6 for ARSEF 5493, and 96.5 for ARSEF 5705, respectively. Mc Clatchie *et al.* (1994) reported that conidia of entomopathogenic fungi must retain high viability and virulence for effective biological control.

Adult mortality: Percentage mortality of adults is shown in Table 5.2. Differences in adult mortality were highly significant between *Beauveria*-treated and untreated aphids. Mean total mortality in untreated adults was significantly lower than adults treated with ARSEF 2883, ARSEF 5493 or ARSEF 5705 ($F = 480.18$; $df = 3, 12$; $P < 0.001$). Differences among *B. bassiana*-treated aphids were not significant, all causing >92% adult mortality. A mortality of 87% (Feng *et al.*, 1990) and >96% (Wang & Knudsen, 1993) have been reported on *D. noxia* treated with *B. bassiana* during an eight and fourteen days period, respectively.

Table 5.2 Mortality of untreated adult *Acyrthosiphon pisum* and those treated with *Beauveria bassiana* during a 10-d period.

Treatment	% Mortality
Untreated	26 ± 4.08a
ARSEF 2883	94 ± 1.96b
ARSEF 5493	94 ± 3.20b
ARSEF 5705	94 ± 1.96b

Means in each column followed by the same letter are not significantly different ($\alpha = 0.05$, *LSD Test* [MSTAT-C]).

Fecundity: *B. bassiana* did not significantly reduce ($P < 0.05$) daily nymphal production of *A. pisum*. Fig. 5.3 indicates average daily nymphal production of untreated and treated adults. Differences in average daily nymphal production were not significant from days 1-9 after treatment. On day 10, there was significant difference between treatments ($F = 4.47$; $df = 3, 12$; $P = 0.03$). These differences observed on day 10 were not necessarily due to *B. bassiana*, because a negligible number of infected aphids recovered (1.4% for aphids infected with ARSEF 2883 and ARSEF 5493 each and 1.6% for aphids infected with ARSEF 5705). Had the isolates affected the daily nymphal production, the percentage of uninfected

nymphs with *B. bassiana* would have been greater than these figures. Furthermore, on day 10, mortality of infected adults was > 90%. It is therefore possible that the difference noted on day 10 was most likely due to the effect of mortality. No signs of infection by any of the three isolates were noted from untreated aphids.

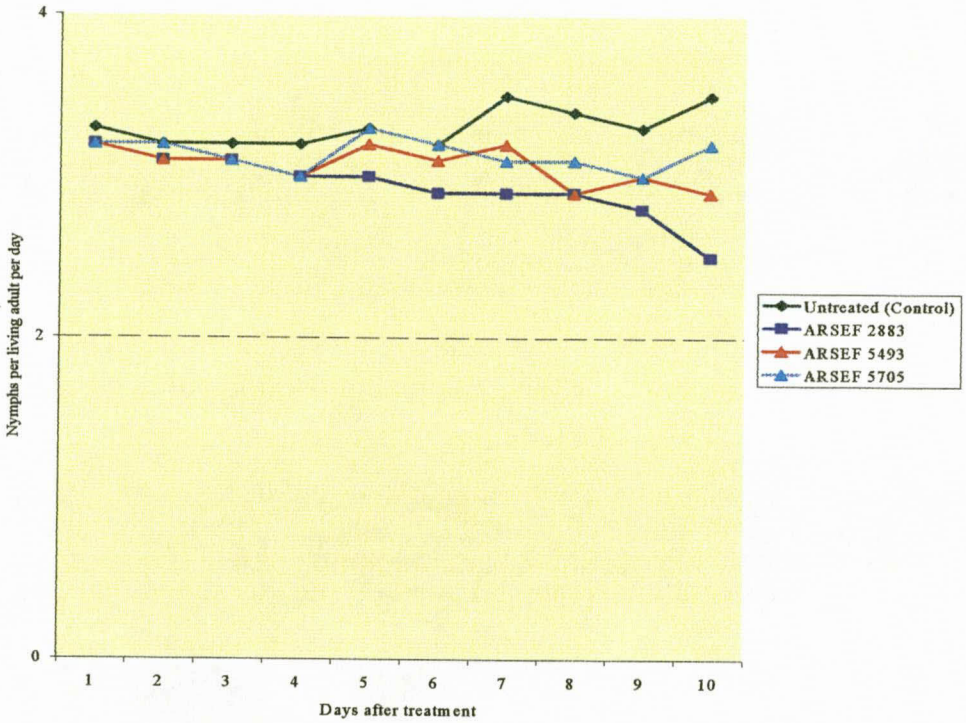

Fig. 5.3 Effect of *Beauveria bassiana* on daily nymphal production of *Acyrthosiphon pisum*

Beauveria bassiana isolates significantly ($P < 0.05$) reduced the total nymph production by living adults ($F = 64.8$; $df = 3,12$; $P < 0.001$). Untreated adults produced significantly more nymphs than *B.bassiana* treated adults (Fig 5.4). These results agree with those of Wang & Knudsen (1993), who reported that *Beauveria* treated *D. noxia* populations were lower than those of untreated aphids. Differences in total fecundity between *Beauveria* treated adults were not significant.

The reduction in total progeny was probably due to mortality of adults infected with *B. bassiana* isolates. These results agree with those of Wang & Knudsen (1993) who reported that *B. bassiana* did not affect the rate of nymphal production of *D. noxia* over 14-d period.

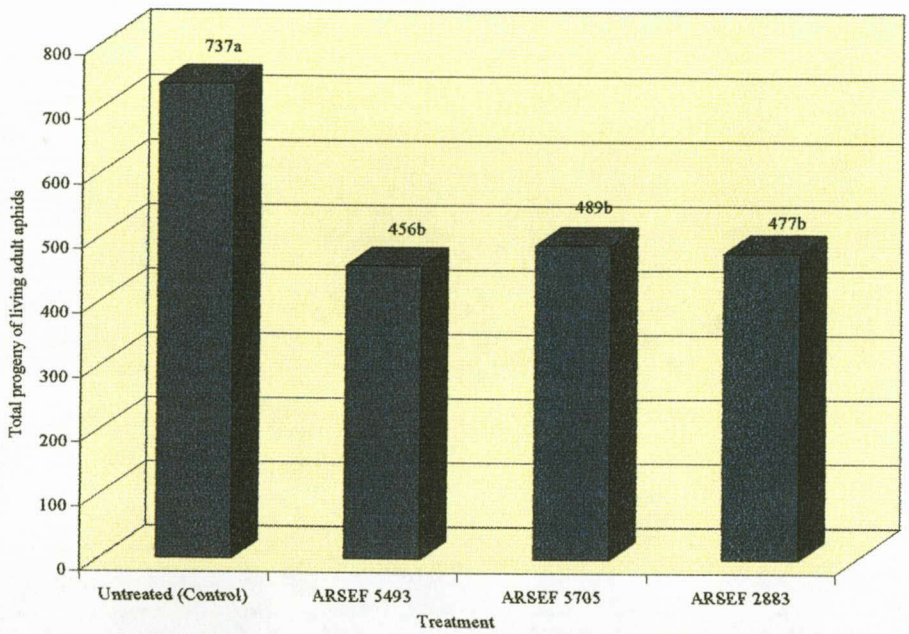

Fig. 5.4 Effect of *Beauveria bassiana* on total progeny of *Acyrthosiphon pisum* over a 10-d period

Although the isolates did not reduce daily fecundity of *A. pisum*, they reduced the overall population by increasing mortality. The capability of isolates in reducing the population of *A. pisum* makes this pathogen effective under controlled environmental condition.

Although biological control of pests has not been practiced as long in greenhouses as field crops (Van Lenteren & Woets, 1988), developments in biological control in this cropping system have been significant. The use of aphid-pathogenic fungi as biological control agents could potentially form part of an alternative strategy for the control of aphid pests, used in combination with low frequent applications of pesticides in IPM programs

(Gray & Markham, 1997). In this point of view, the inculcation of entomopathogenic fungi specifically *B. bassiana* will contribute a paramount importance to integrated pest management of *A. pisum* under controlled environmental conditions through reducing the total population. However, frequent applications might be needed to control new progenies born to infected aphids.

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CHAPTER 6

Evaluation of the botanical product, Neemolin ® and extracts of Wild sering, *Burkea africana* on the fecundity of *Acyrthosiphon pisum* (Homptera: Aphididae)

Abstract

The effects of the botanical product'Neemolin®' and Wild sering, *Burkea africana* were evaluated against *Acyrthosiphon pisum*. A susceptible lentil cultivar 'ILL 8127' was planted (3 seeds per pot) in 1liter plastic pots filled with standard greenhouse soil. After emergence, seedlings were thinned to one. When the seedlings were two weeks old, 'Neemolin' was applied at a rate of 0.5, 1, 1.5, and 2% solution 48hrs before and after infestation in two separate experiments. Water treated plants were also included as a control. Extracts of the wild sering were applied in a separate experiment at a rate of 0.5, 1, 1.5, 2, and 2.5% solution. Water treated plants and plants treated with demeton-S-methyl [Metasystox (I)®] (1.7ml/liter applied as 5ml/plant of the diluted product) were included as a control. Pots were then caged with ventilated nylon organdy to avoid aphid escape. In both experiments, the number of dead and live aphids was counted seven days after infestation. Results of the experiment revealed that application of the botanical product, Neemolin®, significantly ($P < 0.05$) reduced aphid fecundity in all treatments compared with the control. Fecundity was concentration dependent. The interaction between spray time and concentrations was not significant at $\alpha = 0.05$. Application of extracts of wild sering was not effective ($P < 0.05$) against *A. pisum* at all concentrations ($F = 0.29$; $df = 5, 20$; $P > 0.05$). Aphids on plants treated with different concentrations of the extract produced a similar number of progeny to aphids on water-treated plants.

Key words: *A. pisum*, Neemolin®, Wild sering, fecundity

6.1 Introduction

Despite the extensive use of synthetic pesticides after the Second World War, insects remain the main competitors of man for food, especially in developing countries (Schmutterer, 1988). Although pesticides are profitable on the basis of direct crop returns, their application often leads to the pollution of the environment, damage to beneficial insects and wild biota, sudden poisoning of humans and livestock, less biodegradability and the twin problems of pest resistance and pest resurgence. Public awareness of these environmental risks has increased interest in finding alternative pest control methods and products that are as effective as broad-spectrum pesticides, but that are benign to the environment (Spollen & Isman, 1996). In addition to well-known pest management methods such as cultural control, host plant resistance, and biological control interesting approaches are being explored to suppress insect pests using plant extracts to reduce dependence on synthetic pesticides.

Recent studies have shown the importance of natural chemicals as a possible source of non-phytotoxic, systemic, and easily biodegradable alternative pesticides (Singh, 1994; Qasem & Abu-Blan, 1996; Mason & Mathew, 1996). Farmers have been using plant extracts in pest control for centuries. These naturally occurring insecticides in plants are readily broken down in the soil so that they do not pose any threat to the ecosystem. Besides, pesticides derived from plants are inexpensive; and cost effective in developing countries where pesticides are scarce and unaffordable for resource poor farmers.

Among the numerous ingredients of plants studied during the last 20 years, extracts and compounds from the neem tree, *Azadirachta indica*, have attracted the interest of entomologists and phytochemists all over the world (Schmutterer, 1990). Due to their relative selectivity ('stage selectivity') neem products can be recommended for use in many

Integrated Pest Management programs, as it is unlikely that they will cause severe disturbances in ecosystems. Neem based pesticides can make a substantial contribution towards the preservation of biodiversity in ecosystems in spite of the fact that they are not completely safe to all stages of beneficial nematodes, mites and insects (Schmutterer, 1997).

Aphids are economically important pests that are difficult to control due to their mobility, high reproductive ability, and resistance to many synthetic pesticides (Van Lenteren, 1990). Laboratory trials have indicated that the translocation of the active ingredient of neem kernels from the leaf surface into the leaf so as to reach the sucking sites of *Acyrthosiphon pisum* is a fast and effective process (Hummel *et al.*, 1997). Neem based insecticides have been recommended as an effective option for controlling aphids (Schauer, 1984; Patel & Srivastava, 1989; Shmutterer, 1990; Lowery & Isman, 1993). Therefore they should be considered for inclusion in integrated pest management programs (Schmutterer, 1988; Saxena, 1989).

Hence this chapter addressed the efficacy of neemolin® and Wild sering, *Burkea africana* for the control of *A. pisum* on lentil.

6.2 Materials and Methods

6.2.1 The botanical product, Neemolin®

Plants: The susceptible lentil cultivar 'ILL 8127' selected from previous studies was planted in 1liter plastic pots that contained greenhouse soil in a ratio of soil-peat 1:1 v/v.

Infestation: When the seedlings were two weeks old, ten adult (4^{th} instar) pea aphids were transferred from the stock colony using a moistened fine camel-hair brush to each plant 48 hrs before spraying (Post-infestation treatment) or immediately after the neem treated plants are dried (Pre-infestation treatment). The applicator was calibrated before application

to know the exact amount of solution needed to cover the plant. Plants were then treated with neemolin® (0.5, 1.0, 1.5, 2.0%), at 5ml per plant, and with distilled water only (1.06ml/liter) as a control. Spraying was done from all sides with a hand-held mist sprayer to completely cover the foliage to runoff. Each pot was then enclosed in ventilated nylon sleeve cages. The condition of the greenhouse was maintained at 20^0C and 16:8 (L: D) hr for 1 week, after which the number of live and dead aphids was counted. The experiment was conducted as randomized complete block factorial design with seven replications.

Statistical analysis: Count data was square root transformed to normalize the distribution and was subjected to a factorial analysis of variance (ANOVA). Separation of means was determined with Tukey's multiple range test (MSTATC, 1990). Probit analysis was also performed to determine the LC_{50} value (SPSS, 1994).

6.2.2 Wild Sering, *Burkea africana*

Plants: Planting was done according to 6.2.1.

Source and preparation of the extract: Fresh seeds of the Wild sering, *Burkea africana* were collected from Glen, South Africa. Seeds were allowed to dry in an oven at a temperature of 50^0C for 48hrs. Dried seeds were then crushed to powder with food processor, after which time five solutions were made by mixing 0.5, 1.0 1.5, 2.0, and 2.5 g of the powder with 1 liter distilled water, respectively. Prior to application, suspensions were allowed to stay for 4hrs until they dissolve well with water.

Treatment of plants: When the seedlings reach two weeks old, ten fourth - instars of *A. pisum* were transferred from the stock colony using a moistened fine camelhair brush to each seedling. Plants were then treated with suspensions of the seed extract (0.5, 1.0, 1.5, 2.0, 2.5%) at 5ml per plant. Plants treated with distilled water and demeton-S-methyl [Metasystox

(I)®] (1.7ml/liter applied as 5ml/plant of the diluted product) was also included as a control. Calibration of the applicator was done as described in 6.2.1. Each pot was then enclosed in ventilated nylon sleeve cages. Pots were watered every other day and the condition of the greenhouse was maintained at 20^0C and 16:8 (L: D) hr for 1 week, after which the number of live and dead aphids was counted. The experiment was conducted as randomized complete block design with five replications.

Statistical Analysis: Since the data was normally distributed, no transformation was done. Analysis was done using one-way ANOVA as a randomized complete block design at $\alpha = 0.05$ (MSTAT-C, 1990).

6.3 Results and Discussion

6.3.1 The botanical product, Neemolin®

Differences in the time of application were not significant (Table 6.1). The interaction of spray time and concentrations applied was also not significant at $\alpha = 0.05$. However, the influence of treatments on fecundity of *A. pisum* was highly significant (Table 6.1). Applications of neemolin® significantly ($P < 0.05$) reduced aphid fecundity in all treatments compared with the control. Fecundity of aphids was concentration dependent with the lowest fecundity on plants treated with 2% neemolin. However, the difference in fecundity was not statistically significant between aphids treated with 1.5% and 2% solutions of Neemolin, respectively. Similarly, aphids treated with solutions of 0.5% neemolin did not differ from aphids treated with 1% solution in their fecundity. A 1% solution of the Neemolin was the best as it is relatively low dosage, which gave reasonable reduction of nymphs in *A. pisum*.

Table 6.1 Analysis of variance for transformed aphid counts after application of Neemolin®

Source	Degrees of Freedom	Mean Square	F Value	Prob
Replication	6	0.090	0.2943	
Factor A	4	50.021	162.7203	0.0000
Factor B	1	0.014	0.0465	
AB	4	0.050	0.1627	
Error	54	0.307		
Total	69	217.443		

Previous studies have demonstrated that neem extracts effectively reduce the fecundity of aphids. Xie *et al.* (1995) showed that the production of offspring in *Crptilestes ferrugineus* (Stephens) and *Tribolium castaneum* (Herbst) was negatively affected by neem treatments and that the response was proportional to concentration of AZA in the solution.

Lowery & Isman (1996) mentioned that azadirachtin, the active ingredient of neem, might delay the growth of aphid embryos, which would explain how it rapidly inhibits the reproduction of aphids that contain embryos at various stages of development. Furthermore, histological investigations have demonstrated that, in addition to other sensitive tissues, insects treated with azadirachtin have degenerate or improperly developed ovaries and fat bodies (Schluter & Schulz, 1984; Dorn *et al.*, 1987; Schluter, 1987).

In this study, the estimated LC_{50} values are 0.6% neemolin for both pre- and post infestation sprays (Table 6.2). However applications of neemolin prior to infestation did not differ ($P > 0.05$) from post-infestation applications. These results clearly indicate that *A.*

pisum fecundity was not influenced by the contact toxicity of neemolin. Our results agree with those of Lowery *et al.* (1993) who reported that applications of neem seed oil 48hr following aphid infestation did not differ from applications immediately before the infestation for any of the species they tested. According to Schmutterer (1990), neem possesses a relatively weak contact toxicity.

Table 6.2 Fecundity of *A. pisum* following pre and post-infestation foliar applications of Neemolin®

Treatment	Mean number of aphids per plant	
	Pre-I	Post-I
Control	8.6 a (73.1)	8.6 a (73.9)
0.5%	5.6 b (31.1)	5.6 (b) 31.4
1.0%	5.1 bc (25.6)	5.2 bc (26.3)
1.5%	4.5 cd (19.4)	4.3 cd (18.1)
2.0%	3.9 d (14.7)	4.1 d (16.4)
LC_{50}	0.64	0.64
95%CI	0.25 - 0.99	0.25 - 0.99

Means with in the same column followed by the same letter are not significantly different ($P < 0.05$; Tukey's multiple range test)

Pre-I = before infestation: Post-I = after infestation.

Numbers in bracket indicate non-transformed values.

These results clearly indicated that neem insecticides are candidates for inclusion in lentil IPM programs as a selective approach. In conclusion, it can be said that neem products provide a ground for environment friendly pest management with biorational pesticides within the foundation of IPM.

6.3.2 Wild sering, *Burkea africana*

There were no significant differences between treatments ($F = 0.29$; $df = 5, 20$; $P > 0.05$). Aphids on plants treated with different concentrations of the extract produced similar number of progeny as the water-treated plants. All aphids on plants treated with demeton-S-methyl [Metasystox (I)®] had died dead within after 4hrs. Hence data from this treatment were not included in the analysis.

The fact that there is no difference among treatments does not necessarily mean that the extract is not effective against *A. pisum*. The time from dilution to spray was only 4hrs, which might not be enough for the extract to dissolve in water. In some cases, the extracts are boiled to enhance the insecticidal efficiency. It is then applied it after cooling. Therefore, further studies focusing on proper preparation of the extract may be required in order to test it against *A. pisum*.

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CHAPTER 7

Summary

The pea aphid, *Acyrthosiphon pisum* (Harris) is recognized as a devastating pest of lentil, *Lens culinaris* Medikus in Ethiopia. It feeds on plants by inserting a hollow stylet in to the plant tissue to draw out the lifeblood of the plant. Known symptoms are yellowing, stunting and death of the plant, respectively. Reportedly, this pest causes downright crop failures to lentil production.

Several routine control measures have been practiced so far. Despite this, the loss due to this pest is dramatically high every year sometimes causing total crop failures. The aim of this study was to investigate aspects of integrated pest management that will allow:

1. Knowledge on the biology of this pest
2. Selection of resistant cultivars
3. Identification of mechanisms of resistance harboured by resistant cultivars so as to use them in a breeding program
4. 'Better' management of *A. pisum* by reducing the impact of this pest on lentil production in consideration with ecological risks.

Preliminary screening for plant resistance allowed identification of the relative resistance or susceptibility of each entry tested. The mechanisms of resistance study indicated that French Indigo had the highest antibiotic plant resistance to South African strain of *A. pisum*. Antixenotic resistance was also found in this entry. Spanish Brown showed strong antixenotic, tolerance and antibiotic plant resistance. Verdina also expressed antixenotic and antibiotic components of resistance. LC 660615L has more of antibiotic property. Neither antixenotic nor tolerance is found in it. Brewer appeared to have the lowest

host plant resistance of all. This cultivar may have some antixenotic property however, neither antibiosis nor tolerance component of resistance was noted.

Application of *Beauveria bassiana* as a biological control agent also proved the potential of this pathogen in reducing the population of *A. pisum*, at the same time reducing environmental risks.

The use of Neem seed oil was also effective against *A. pisum* even at lower dosages. Furthermore, within the framework of integrated pest management (IPM), Neem seed oil could serve a sustainable solution to the pesticide problem.

The results of this study will contribute towards a better IPM program, especially in light of increasing productivity and profitability of lentil without endangering the environment. Moreover, the resistant entries reported in this study will contribute as a potential parent in breeding programs. However, as races differ in different geographical regions, exposure of the entries to various *A. pisum* races of varying geographical places should be taken in to consideration.