# LABORATORY AND FIELD EVALUATIONS OF PROPOLIS AS A PLANT PROTECTIVE AGENT

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# *ABSTRACT*

Propolis is a plant derived resinous substance with known antibiotic properties. Laboratory and field trials were carried out in 1989/90 to evaluate propolis for control of insects and diseases in horticultural systems. Field trials were carried out in the organic block of Levin Horticultural Research Station. Ether extracts of propolis in agar (10, 100 1 000 and 10 000 ppm) were screened against 20 plant pathogenic fungi. Radial mycelial growth from fungal plugs were measured daily. Propolis inhibited the growth of all fungi tested although the sensitivity of fungi to propolis varied. The EC50 was between 100 and 10 000 ppm for all species with complete inhibition at 10 000 ppm in 16 species. Propolis collected from different geographic locations had different activity. There was less antifungal activity in water extracts than in ether extracts of propolis.

Ethanol, surfactant and ethanol extracts of propolis were sprayed on cucumber plants weekly in a glasshouse. Weekly estimates of powdery mildew cover (*Erysiphe cichoracearum*) for 5 weeks were analysed. Foliar spray applications of 1% propolis extract reduced powdery mildew cover from 84.5% in the untreated plants to 33.4% in the treated ones.

Eight treatments were tested on a 10 day spray calendar on zuccinis. Assessment for powdery mildew cover was made on four occassions. The number of harvested fruit from each plant were recorded. A 1% ethanol extract of propolis reduced powdery mildew only until the second assessment, 39% vs. 60% cover in the controls. The fruit number was not affected by treatments.

Late blight of tomatoes (*Phytophthora infestans*) in the field was not affected by foliar sprays of 1% propolis extract. Radish seeds treated with a seed dressing of 36% propolis extract were not protected against (*Pythium ultimum*) in agar petri plate trials

Laboratory screening of propolis against light brown apple moth (*Epiphyas postvittana*) and green peach aphid (*Myzus persicae*) did not indicate sufficient activity to be used in crop protection.

In conclusion propolis showed some antifungal activity in laboratory trials. Successful applications in the field using the methods evaluated here however would require concentrations of raw propolis that are both impractical and uneconomic. The potential for use of propolis in plant protection is likely to come from further chemical analysis, with identification of active components and their possible synthesis.

Key words: propolis, fungicide, insecticide, *Phytophthora infestans,* late blight of tomatoes, *Erysiphe cichoracearum*, powdery mildew of cucurbits, *Pythium ultimum*, damping off, light brown apple moth (*Epiphyas postvittana*), green peach aphid (*Myzus persicae*).

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# CHAPTER 1

# *INTRODUCTION*

Management of plant pests and diseases is necessary in most agroecosystems to obtain an acceptable quantity and quality of yield. The development of synthetic organic pesticides after World War II allowed for increased yields while reducing the apparent need for cultural and biological controls. The resulting dependence on pesticides for control has created technical problems of resistance, resurgence and secondary pest outbreaks, and cultural problems of environmental and health hazards. These problems have resulted in the term 'the pesticide crisis' and have caused an upsurge of interest in other control strategies including the use of naturally occurring pesticides (Perkin 1985). These include microbial, plant and animal derived products which are seen as less dangerous and generally more biodegradable than synthetic pesticides (Pimental 1985).

Research into natural products may produce a natural pesticide or a model on which to base synthetic pesticides. Natural pyrethrum and the synthetic pyrethroid analogues respectively exemplify both these cases. Pyrethroids have the desirable characteristics of being readily biodegradable, being selective in their action, requiring only low application rates and providing a quick knock down effect. These are the type of qualities sought in new pesticides (Todhunter 1985).

Other problems associated with the pesticide crisis can be minimised by integrated pest management where the application of any pesticide occurs only when necessary with respect to other control mechanisms.

Organic farming uses integrated pest management and promotes a holistic approach to agroecosystem management. However, fundamental to organic growing standards, is production without the use of chemically synthesised compounds except under exceptional circumstances. Naturally occurring pesticides may therefore be important for control in organic farming where pests are a problem.

This study investigated the potential of propolis as a pest control agent. Propolis, a product from beehives, has known antibiotic properties and in these trials was tested against plant pathogenic fungi in the laboratory and in the field and against insect pests in the laboratory.

CHAPTER 2

# *LITERATURE REVIEW*

## *2.1 Review of pest and disease organisms*

The pests and diseases studied in the outdoor trials of this research were those which are common in crops in the Levin area.

***Diseases***

For each plant disease studied in this trial the classification of the causal species, its epidemiology, etiology, symptoms and the methods used for control in horticultural systems are given.

### *2.1.1 Powdery mildew of cucurbits*

Pathogen:

| | | |
|---|---|---|
| Class | | Ascomycetes |
| Order | | Perisporiales |
| Family | | Erysiphaceae |
| Subfamily | | Erysipheae |
| Species and genus | or | *Erysiphe cichoracearum* DC<br>*Sphaerotheca fuliginea* (Schled. ex Fr.) Poll. |

Etiology and Epidemiology

Outbreaks and development of powdery mildew in cucurbits are favoured by dry atmospheric conditions, moderate temperatures, low light intensity, fertile soil and succulent plant growth (Yarwood 1957). Glasshouses are consequently conducive to success of the pathogen particularly where continuous cropping occurs. Microclimate in the field also greatly affects the incidence and severity of disease with canopy structure being a key determinant.

The ascospores and conidia are spread by wind. They have a high water content and are therefore capable of germination in the absence of moisture at the plant surface and a relative humidity of less than 20%. Under suitable

conditions the spores germinate within two hours producing germ tubes and appresoria. After four days conidiophores are formed with the full life cycle taking 5-6 days (Stillerly 1956). *E. cichoracearum* is an ectoparasite as the haustorium absorbs food material from its host allowing growth of mycelium over the leaf surface (Brien and Dingley 1956).

Symptoms

The visible symptoms of powdery mildew on cucurbits progress from tiny, white round superficial spots to a white powdery covering over much of the leaf and stem surfaces. Young leaves and severely infected mature leaves and stems may become chlorotic and die. Fruit are generally free of visible infection and any reduction in yield is dependent on the time and severity of disease development. Late fruit may not mature and may be small and misshapen (Walker 1952).

Control

Prior to the 1950s, sulphur was the main material used for chemical control of powdery mildew. *E. cichoracearum* is vulnerable to elemental sulphur throughout its life cycle, except the cleistothecial stage. Application of 4-5 kg $ha^{-1}$ of sulphur at 10-14 day intervals was a common field control strategy. The sulphur acts by selective toxicity with *E. cichoracearum* being more sensitive than the host plant. This however causes problems with sulphur sensitive plants such as a cantaloupe and cucumber. Consequently in the 1950s organic fungicides that could be used on sulphur sensitive species were developed. The nitrocompound dinocap, Karathane$^{R}$ for example, was the first to be introduced and had both eradicant and protectant action. Now it is marketed only for its protectant property (O'Connor 1990).

Triadimefon and benomyl are more recent products that are both systemic and protectant in their action. Bayleton$^{R}$ and Benlate$^{R}$, are trade preparations of these chemicals. Resistance build up by the plant pathogens against these chemicals is common. Their use should therefore be kept to a minimum by timing applications according to climatic conditions and by alternating with other products when frequent applications are required for protection.

Economic importance

Powdery mildew rarely directly affects fruit quality. It is only economically important when the disease reduces effective leaf area to an extent that it results in reduced yield.

### *2.1.2 Late blight of tomatoes*

Pathogen:

| | |
|---|---|
| Class | Phycomycete |
| Order | Pernosporales |
| Family | Pythiaceae (water moulds) |
| Genus | *Phytophthora* |
| Species | *infestans* |

Etiology and Epidemiology

Germination of spores of *P. infestans* requires moisture so the disease is favoured in wet seasons and with cool nights and warm days when dew forms. The spores are killed with dry atmospheres and with temperatures greater than 27°C. The optimum temperature for germination is 10-15°C. After successful infection *P. infestans* produces copious mycelium which branches out through host tissue (characteristic of Pernosporales). Sporangia are produced on special branched mycelia (sporangiophores) that arise through stomatal openings on lower surfaces and margins of lesions. These sporangia produce zoospores which may then be liberated and dispersed to adjacent plants by rain splash, wind or insects. *P. infestans* may survive between crops in plant refuse in the soil (Brien and Dingley 1956).

Symptoms

The first symptoms appear as greenish/brown, irregular, water soaked patches on leaves and leaf stalks. As the mycelium moves through the host tissue these areas enlarge and darken. During wet weather sporulation occurs on these lesions and symptoms spread to the stems and fruit. The fruit lesions develop

from greenish brown to brown with definite margins. When conditions are warm and wet for prolonged periods at the plant surface the infected leaves are killed and within a few days the whole plant may die (Brien and Dingley 1956).

Control

Prior to the 1970s control of late blight of tomatoes and potatoes was achieved chemically using copper, principally Bordeaux mixtures ($CuSO_4$ and hydrated lime). This acts as a protectant on the surface of the plant by inhibiting germination of the spores. Various copper formulations have been developed, such as CuOCl, but with CuOH being the most common in usage now. New formulations, such as those marketed as Kocide$^R$ and Champ$^R$, aim at reducing the particle size to minimise the amount of copper required to provide a protectant coating against the pathogen.

In 1977 metalaxyl was introduced for the control of late blight. With systemic action it was advantageous over the purely surface protectant character of copper. However resistant sporangia built up rapidly under blight favourable conditions with the resistant isolates being especially competitive over the susceptible ones (Davidse *et al* 1985). This initiated the implementation of new control strategies for foliar diseases based on formulated mixes (Staub *et al* 1984). Ridomil$^R$ MZ 72WP is one such formulation mix which consists of metalaxyl and mancozeb (O'Connor 1990). Use of this systemic and protectant mix reduces the selection pressure from each chemical.

Chemical disease control in organic systems relies largely on the use of copper as a protectant. The copper must cover the plant surface whenever conditions are conducive to infection. However high levels of application may be harmful to the soil biota. There have been several reports of copper based materials causing a reduction in earthworm populations. Cluzeau and Fayolle (1988) for example compared vineyards that did and did not apply copper sprays. The sprayed orchards had a consequently higher level of soil copper and had virtually no earthworms whereas those with low soil copper levels had normal earthworm populations. Wei-chun Ma (1984) reported that additions of copper based materials to soil decreased cocoon production and may consequently seriously affect earthworm populations.

Management to minimise conditions that are optimal for *P. infestans* minimises the requirement for chemical control. The humidity and moisture at the plant surface can be reduced by encouraging good air flow within the crop. This is achieved by removing the lower leaves and pruning to produce an open canopy, training onto wires for example.

Tomatoes and potatoes should not follow potatoes in a rotation as the potato tuber and plant debris are common overwintering hosts for the pathogen. Likewise tomatoes should not be planted next to potatoes as tubers and the potato crop act as a source of inoculum for disease spread (C.M.I. 1985).

Economic importance

As a result of the direct effect on fruit quality and indirectly on the yield *P. infestans* has the potential to cause high economic losses.

### *2.1.3 Storage rot of kiwifruit*

Pathogen:

| | |
|---|---|
| Class | Fungi imperfecti |
| Order | Hyphomycetes |
| Family | Monilliales |
| Genus | *Botrytis* |
| Species | *cinerea* |

Etiology and epidemiology

*Botrytis cinerea* causes kiwifruit to rot when stored at 0°C. Two stages of infection have been suggested by Pennycook (1984). At blossom time dying petals act as a food source for *B. cinerea* which can consequently grow and produce masses of spores. Inactive symptomless infections (quiescent infections) can become established in areas where the petals are attached to the newly formed fruit. About six months later these quiescent infections may be reactivated and rot symptoms develop.

The second suggested time of infection is at harvest when inoculum enters the fruit through the harvest wound. Beever *et al* (1984) suggest that this is the main source of infection.

Symptoms

The external symptoms of the storage rot are a conspicuous darkening and softening of the fruit starting at the calyx end. As the rot progresses through the fruit a faint pinkish fawn discolouration of the skin may develop. Later a thick white hyphal mass may appear with sporulation and nesting, or infection of neighbouring fruit may occur. Generally the first rots are visible after 3 weeks of storage after harvest, most are present after 4-6 weeks while some may not develop for 3-4 months.

Economic importance

Storage rot caused by *B. cinerea* was virtually unknown in kiwifruit in New Zealand before 1978, but by 1984 infection levels were commonly reaching 1% (Pennycook 1984). It is now considered a disease of major economic importance. The infection may spread to give 25% loss of fruit and any presence of storage rot can have a major detrimental effect on New Zealand's high quality export image.

Control

Control of the storage rot presently relies on chemical sprays at blossom and at harvest to reduce inoculum levels. A post harvest dip to inhibit entry of the pathogen through the harvest wound is not permitted because of residue restrictions on export fruit.

The dicarboximides vinclozolin and iprodione, marketed as Ronilan$^{R}$ and Rovral$^{R}$ are the two recommended fungicides to be sprayed at flowering and one day before harvest. They both have eradicant activity but as resistance may occur their use should be restricted to only the two sprays in the season (O'Connor 1990).

### *2.1.4 Damping off of seedlings*

Pathogen:

| | |
|---|---|
| Class | Phycomycete |
| Order | Pernosporales |
| Family | Pythiaceae (water moulds) |
| Genus | *Pythium* |
| Species | *ultimum* |

Etiology and epidemiology

Like *Phytophthora infestans*, *P. ultimum* is a water mould and therefore requires water to complete its life cycle. *P. ultimum* survives as oospores and can probably subsist as a saprophyte or as a low grade parasite on fibrous roots (Walker 1952). *P. ultimum* can produce copious filamentous mycelia which ramify through cells of the plant. Asexual sporangia form and produce zoospores rapidly and hence given a food source the disease may quickly develop.

Symptoms and economic importance

The failure of seedlings to emerge is the most common symptom of *P. ultimum* attacking germinating seeds. This can cause major losses in seeds and is a particular problem in organic growing where standards require that seedlings are produced without the use of synthetic chemicals.

Control

In conventional seedling production *P. ultimum* is effectively controlled using fungicide seed dressings or slurries. Apron$^{R}$, for example, is a metalaxyl based systemic chemical that penetrates the seed coat and protects the emerging seedling from oomycete damping off pathogens (O'Connor 1990).

*Insect pests*

Laboratory trials were carried out on two important horticultural pests, the light brown apple moth, *Epiphyas postvittana*, and the green peach aphid, *Myzus persicae*. Both these insects are major pests and the problems involved in their control are representative of those involved with many of New Zealand's insect pests. The following gives a background on their classification, life history, economic importance and control.

### *2.1.5 Light brown apple moth*

| | |
|---|---|
| Class | Lepidoptera |
| Family | Tortricidae |
| Genus | *Epiphyas* |
| Species | *postvittana* |

Life history

*E. postvittana* overwinters as larvae on ground cover, mummified fruit or on evergreen host plants. The adults fly and lay eggs in early spring on leaves in fruit crops. The new larvae may colonise new leaves or new plants by suspending themselves on silk threads and moving in wind currents. Young larvae feed on the lower leaf surface and shelter themselves by spinning a web. Older larvae web leaves together or to fruit or roll the edge of leaves over (characteristic of leaf roller species). Pupation takes place on foliage or beneath the tree. Several generations occur each season depending on climate (Penman 1984).

Economic importance

*E. postvittana* is considered a major orchard pest in New Zealand because of the fruit and foliar damage it causes (Singh *et al* 1984) and its wide host range. Larvae remove the epidermal layer of fruit causing significant cosmetic

damage. This downgrading of fruit is amplified by secondary infections or by the fruit forming scar tissue over the feeding area, as in kiwifruit. Flowers, buds and growing tips may also be damaged by feeding larvae (Penman 1984).

Control

For many export markets there is nil tolerance for leaf roller damage in fruit. This emphasises the necessity for accurate timing of insecticide applications as only the young unsheltered larvae are exposed to chemicals. Use of sex pheromone traps for monitoring populations could improve the precision of application (Suckling *et al* 1984).

Leaf roller populations can be reduced by removing mummified fruit, grazing ground cover over winter and using shelter trees that are not favoured as host plants (Penman 1984).

Broad spectrum organophosphates are the main group of chemicals used for leafroller control, azinphos-methyl sold as Gusathion$^{R}$ for example (O'Connor 1990). Their broad spectrum activity limits their use in integrated pest management and their general toxicity reduces the desirability of their use.

In organic systems, preparations based on *Bacillus thuringiensis*, (for example Thuricide$^{R}$) may be used for control of lepidopterous pests including leaf roller in apples. Thuricide$^{R}$ does not harm ladybirds, lacewings, syrphid flies, bees, wasps or predatory mites and has only low mammalian toxicity (O'Connor 1990). However it is not systemic and must be ingested and therefore must be present as a deposit on the surface of the leaves where the larvae feed.

### *2.1.6 Green peach aphid*

| | |
|---|---|
| Class | Hemiptera |
| Family | Aphididae |
| Genus | *Myzus* |
| Species | *persicae* |

Life History

*M. persicae* overwinters in cooler areas of New Zealand either as eggs on a primary woody host or as winged forms on a secondary host. The eggs hatch as

wingless forms in spring and as numbers increase winged forms are produced that migrate to secondary hosts. Winged and wingless forms are produced throughout summer by parthenogenetic reproduction (Penman 1984).

Economic importance

*M. persicae*, like other aphids may cause direct plant damage by foliar feeding resulting in yellowing, wilting and distortion. Its more economically damaging effect however is the transmission of many plant viruses (Fenemore 1984)

Control

Control of virus transmission is difficult as a result of the large numbers of aphids that may occur throughout the growing season and the short amount of feeding time required in some cases for virus transmission. Systemic insecticides provide some protection but continuous protection is required to inhibit virus transmission. As in leafroller control most of the chemicals used for aphid control are broad spectrum organophosphates, many with systemic action.

Aphid control in organic growing relies on biological control and natural substances such as derris dust, garlic, soap solutions, nettle, potassium permanganate, and natural pyrethrum sprays.

## *2.2 Propolis*

### *2.2.1 Definition*

Propolis is the material used by bees to seal hive walls and to strengthen the borders of their combs. The word is derived from the Greek :

pro- for or in defence
polis- the city, or the hive (Ghisalberti 1979).

### *2.2.2 Composition*

Propolis is made by bees from the sticky plant substances on the surface of woody plants. The bee gathers these substances in its mandibles and may carry 10 mg in the pollen baskets on its hind legs back to its hive (König 1985). In the hive the plant substances are mixed with bees wax and the resulting propolis added to the hive. The plant substances collected may include different types of secretions, such as lipophilic substances, mucilage, gum, oil and possibly wax, and exudates, largely resin and latex (Walker and Crane 1987). Manufactured products such as paint, bitumen and mineral oils have been used by bees where plant sources were not available (König 1985).

Propolis generally consists of 55% balsams and resins, 30% waxes, 10% etheral oils and 5% pollen (Brown 1989). The balsams and resins are largely derivatives of flavins, vanillins, chrysin and allied compounds, with aromatic unsaturated compounds like caffeic and ferulic acids. 149 constituents of propolis have been reported of which 38 are flavonoids, 14 derivatives of cinnamic acid and 12 derivatives of benzoic acid. Eleven other groups have been listed including terpenes and sesqui terpenes, alcohols and hydrocarbons (Walker and Crane 1987).

The aromatic compounds found in propolis are also common in plant sources (Ghisalberti 1979). The roles of these secondary compounds in plants are still unclear although there is increasing evidence of their role in growth, development and particularly defence (Vickery and Vickery 1981). There are many examples of phenolic compounds inhibiting growth or spore germination of particular fungi and of compounds acting as deterrents, repellents or toxins to insects (Harborne 1988). Such compounds are found at the plant surface

where they may provide a chemical barrier to invading organisms. For example flavonoids isolated and identified from the leaf surface of *Helichrysum nitens* and *Erythrina berteroana* were found to have significant antifungal activity (Tomas-Barberan *et al* 1988). Other plant defence compounds, phytoalexins, are produced specifically in response to invasion of an organism and accumulate at the site of infection (Harborne 1987). Plant defence compounds are consequently relatively abundant at wound sites and at other points vulnerable to attack such as young buds and bud scales, the areas from which bees collect exudates for propolis.

Some of the compounds that have been identified in propolis such as quercetin flavone and cinnamic acids are toxic to insects and may act as a feeding deterrent (Eischen and Dietz 1987). Corsi (1981) found that the essential oils identified in propolis were typical of those from likely source plants in the area. For example vanillin, eugenol and borneol are typical essential oils of coniferous plants and juniper (*Juniperus* sp.) and were found in propolis collected from areas with this flora.

The compounds used in plant defence have a rapid turnover time as the plant breaks the compounds down or converts them to other secondary compounds. The concentration in tissues therefore varies greatly depending on age of the tissue, stage of life cycle, vulnerability to attack and time of year (Vickery and Vickery 1981). This will consequently affect the concentration and types of compounds found in propolis.

The existence or extent of transformation of the plant substances by the bee as it produces the hive propolis is unknown. By using gas chromatography - mass spectrometry analysis on *Populus* x *euroamericana* bud exudate and propolis Greenaway *et al* (1987) confirmed the bud exudate as a primary source of propolis. Other compounds identified were derived from wax secreted by the bees and materials such as sugars that may have been accidentally introduced by the bee during manufacture of propolis or during subsequent passage of the bees over the propolis. One glucoside was found in abundance in the bud exudate but not in any propolis sample. It was suggested that this compound underwent enzymic hydrolysis by the bees, either during collection from the poplar or during addition of beeswax to the propolis.

New compounds are still being identified in propolis and in possible propolis sources. Bankova *et al* (1989), for example, isolated and elucidated the structure of two esters of caffeic acid and two esters of ferulic acid with isomeric pentenyl alcohols from two species of poplar and from propolis.

### *2.2.3 Propolis in the bee hive*

Bees make use of both the physical and antimicrobial properties of propolis. Bees wax gives the main physical support in the hive as it is estimated to be eight times stronger and seven times stiffer than propolis (Adey 1986). The elasticity and strength of propolis however is used to produce a thin layer on the internal walls of the hive or any cavity the bees may inhabit (Ghisalberti 1979). Propolis is used to block holes and cracks, to repair combs, to strengthen the thin borders of the comb and for making the hive entrance weather tight and easier to defend. Propolis is used with wax to cover hive invaders that are too big for the bees to remove from the hive, wax moths or snails for example. Antimicrobial properties may keep the hive free of fungi and bacteria which would be expected to thrive in the humid environment of the hive.

### *2.2.4 Plant sources*

The range of plant species used for propolis contributes to its complex and variable composition (Eischen and Dietz 1987). In the northern temperate zone poplar, elm, birch , alder, beech, horse chestnut and conifer are accepted as the main sources of propolis (Ghisalberti 1979). Eucalypts and introduced poplars are important sources in Australia and collection from the native grass trees, *Xanthorrhea* sp. may also occur (König 1985).

The trigger for propolis production is unknown. In Italy the main collecting season is spring, in eastern and northern Europe mid summer and in USA late summer and autumn (Koenig 1985). Collection may be dependent on the availability and softness of plant exudates, most likely favoured by warmer conditions.

Yield of propolis varies between hives and between years. Ghisalberti (1979) suggested some colonies may produce 150-200 g/year.

### *2.2.5 Uses of propolis*

Pharmaceutical uses

Records of the use of propolis date back to at least 300 BC when it was used for its resinous and glue like properties (Ghisalberti 1979). More recently its antibiotic properties were used in folk medicine. However it is only in the last 40 years that its composition, pharmacological properties and commercial uses have been researched. Much of this work has been carried out in Eastern European countries and therefore is of limited availability to western English speaking people.

There have been many reports on the antibiotic properties of propolis. Kivalkina (1948) produced the first published research on the activity of propolis on a range of bacteria including *Streptococcus aureus*, the typhoid bacillus. More recent research includes that of Mlagan and Sulimmovic (1982) who demonstrated the inhibitory effect of propolis on *Bacillus larvae in vitro* and Anastasiu (1978) the inhibitory effect on *Pseudomonas aeruginosa*. This latter work highlighted the greater susceptibility to propolis of gram positive than gram negative bacteria. Lindenfelser (1967) tested a range of propolis samples and found most demonstrated both antibacterial and antifungal activity.

The antibiotic, styptic, astringent, antiinflammatory and anaesthetic properties of propolis have been exploited for pharmacological uses. Examples include treatment of ear and respiratory infections, ulcers, wound healing and skin tissue regeneration (Ghisalberti 1979). Antiviral activity has also been reported for propolis. König and Dustmann (1985) suggest this may be attributed to caffeoylics, a family of antiviral active compounds found in propolis. These compounds have activity against avian *Herpes* viruses and research is continuing into viruses from mammalian hosts.

Propolis is generally regarded as harmless to humans. However cases of hypersensitivity have been reported causing severe allergic reactions of the skin. Esters of caffeic acid have recently been identified as the responsible contact allergens (Stüwe *et al* 1989).

Uses in agriculture

The above antibiotic properties of propolis suggests the possibility of the use of propolis as a plant protective agent. The range of fungi found to be sensitive to propolis by Lindenfelser (1967) included 25 phytopathogenic species. Garofolo (1987) reported propolis in combination with sulphur to be highly effective against a wide range of pests and diseases *in vivo* (Table 2.2). The propolis / sulphur treatment, treatment 1, and the propolis / sulphur followed by Thioram, treatment 2, both gave similarly good control of bacterial and fungal pathogens, with treatment 1 also having good control of the insect pests. Both the propolis treatments gave better control than the synthetic pesticides in all cases.

A comparison was made between the efficacy of these preparations on plants grown in an organic system to those grown in a conventional system. The plants grown in the organic system responded quickly with rapid elimination of the pest problems. In contrast the plants in the conventional system responded only slowly and eventually chemical control was required.

### *2.2.6 Extraction of propolis*

Propolis is obtained from the hive by scraping the inner covers and top bars (Wright-Sunflower 1988). Various extractants have been used for obtaining the active ingredients from propolis. Meresta and Meresta (1982) found the best extraction method for activity against bacteria was a solvent mixture including methyl alcohol, ether, acetone and chloroform. Ethanol has often been used, but Meresta and Meresta found this to be less effective and more expensive than other organic solvents such as ethyl ether, ethyl acetate and methylene chloride.

Mlagan and Sulimanovic (1982) found both aqueous and ethanol extracts to be effective against *Bacillus larvae in vivo*, with the ethanol extract having a slightly greater activity.

**Table 2.2** Activity of propolis plus sulphur against plant pests and diseases (Garofolo 1987)

| Pest | Host plant | Percent efficiency Trt 1* | Trt 2* | Trt 3* |
|---|---|---|---|---|
| Bacteria | | | | |
| *Aplanobacter michiganensis* | tomatoes (plant and fruit) | 98.3 | - | 0 |
| *Micrococcus populi* | poplar | 87.9 | - | 0 |
| *Pseudomonas savastanoi* | olives | 98.8 | - | 0 |
| Fungi | | | | |
| Oomycetes | | | | |
| *Phytophthora infestans* | potatoes and tomatoes | 85.9 | 91.8 | 65.3 |
| *Plasmopara viticola* | grapevines | 87.7 | 92.5 | 77.4 |
| Ascomycetes | | | | |
| *Taphrina deformans* | peach (foliage) | 89.9 | 95.7 | 75.8 |
| *Anntenaria elaeophila* | olive (foliage) | 91.2 | 97.6 | 69.9 |
| *Penicillium digitatum* | citrus (fruit) | 97.8 | - | 62.8 |
| *Sphaerotheca pannosa* | peach and rose (foliage) | 86.5 | 88.9 | 79.6 |
| *Uncinula necator* | grapevines | 89.3 | 91.7 | 78.9 |
| *Microsphaera lonicera* | | 84.2 | 89.5 | 67.5 |
| Basidiomycetes | | | | |
| *Puccinia* sp. | tarragon (foliage) | 88.6 | 90.8 | 69.8 |
| Deuteromycetes | | | | |
| *Phyllosticta populina* | poplar (foliage) | 88.6 | 91.5 | 74.6 |
| *Botrytis cinerea* | grapes | 89.7 | 92.8 | 67.5 |
| *Cycloconium oleaginum* | olive (foliage) | 85.9 | 89.7 | 79.8 |
| Insects | | | | |
| *Myzus persicae* (peach aphid) | brassicas | 5.8 | - | 79.7 |
| *Macrosiphum rosae* (rose aphid) | roses | 97.5 | - | 77.3 |
| *Eriosoma lanigerum* (woolly apple aphid) | apple | 96.7 | - | 65.9 |

*Treatment 1 : 150 grams hydro-alcoholic solution of propolis and 250 grams sulphur in 100 litres of water; applied at 10-15 day intervals around sunset.

*Treatment 2 : treatment 1 followed by 5-10 grams Thioram (a ramic acid-sulphur mix rich in micronutrients) at 25,50,100 $kgha^{-1}$ depending on the foliage development.

*Treatment 3 : application of dithiocarbamate or synthetic pesticide for pathogen or insect attack respectively, applied according to label instructions.