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The effectiveness of *Solanum panduriforme (Mey*) based extracts on the cabbage aphid, *Brevicoryne brassicae (Linnaeus*) on brassicas

BY

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DECLARATION

I Mary Louis Mhazo declare that the thesis, which I hereby submit for the degree of Doctor of Philosophy at the University of Venda, is my own work and has not previously been submitted by me for a degree at this or any tertiary institution.

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ABSTRACT

Brassicas are important vegetable crops grown for home consumption and market gardening in eastern and southern Africa. However, productivity is affected by aphids, through both direct feeding and disease transmission. Botanical insecticides have potential to control the aphids, but so far few plants have been evaluated for use on brassicas. This study was conducted to evaluate the effectiveness of *Solanum panduriforme* to control aphids on brassicas. Botanical extracts from three parts of *S. panduriforme* were assessed for their insecticidal effects on the cabbage aphid, *Brevicoryne brassicae*. The extracts from leaf powder (LP), ripe berry powder (BP), fresh ripe berries (RB) and fresh unripe berries (UB) were extracted with four solvents; water, ethanol, hexane and diethyl ether, using homogenisation, maceration and solvent-assisted / sequential extraction methods. The effectiveness of the extracts was determined by laboratory bioassays as well as by plant assays in the screen house and under field conditions. The experiments were replicated three or four times depending on the assays and the design used was completely randomized design (CRD). The immature (LP and UB) plant parts were generally more effective than the mature (BP and RB) plant parts, with mortalities ranging from 100 % down to 40 % respectively depending on assays. Ethanol extracts were more effective than aqueous extracts (LP 96% and 63%; BP 96% and 64%; RB 100% and 64%; UB 100% and 90%). The dried crude extracts from hexane were more effective than di-ethyl ether extracts. The group chemical analysis indicated presence of alkaloids in the berries (BP, RB and UB), which were absent in the leaves (LP). Phenolic compounds and flavonoids were present in all the extracts (LP, BP, RB, and UB). Saponins were present in the fresh parts (RB and UB). The results show how the locally available *S. panduriforme* plants can be used as an aphicide to control aphids on brassicas. Farmers can directly prepare an easy and cheap botanical insecticide from leaf powder and unripe berries with maceration and homogenisation techniques using water as the extraction solvent. The berries can be further investigated for development of a commercial botanical insecticide.

Key words: *A*phicide, bioassays*,* brassica*, Brevicoryne brassicae,* effectiveness, extracts, mortality, *Solanum panduriforme*,

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PREFACE

Brassicas such as cabbage, rape (indigenous and exotic types) and kale are important vegetable crops grown for home consumption and market gardening in eastern and southern Africa but their production is hampered by major insect pests which include aphids. The use of synthetic insecticides to control the insects is associated with environmental and health hazards. Botanical insecticides have been suggested as an alternative to the dangerous synthetic insecticides but their use is limited due to lack of standardized extraction methods and insufficient knowledge on their use which result in variable effectiveness. *Solanum panduriforme* is sometimes used for veterinary, medicinal and plant protection purposes but there is dearth of information to support its effectiveness. The information gap is crucial for the plant to be adopted for use by more farmers or for synthesis into a standardised commercial botanical insecticide by industry.

The aim of this research was to evaluate effectiveness of extracts from *Solanum panduriforme* leaves and berries on the mortality of the cabbage aphid, *Brevicoryne brassicae.* The objectives were to investigate the effects of aqueous and organic solvents extracts against aphids and to identify the bioactive compounds.

Chapter 1 provides a background of the studies and how *S. panduriforme* can be used as an alternative to synthetic insecticides. The research problem and justification is outlined together with the research questions and hypothesis. The aim of the research was to evaluate the effectiveness of the extracts from leaves and berries of *S. panduriforme* against the cabbage aphid on brassicas. The objectives were to investigate the effects of the extracts using repetitive bioassays in the laboratory and plant assays in pots and in the field and also to identify the bioactive compounds from the leaves and berries using qualitative phytochemical analysis.

Chapter 2 is a literature review to outline the importance of brassicas, to highlight aphids as major insect pests in brassica production and how they can be controlled using botanical insecticides as an alternative to synthetic insecticides. The characteristics of *S. panduriforme* which makes it a suitable alternative to synthetic insecticides are reviewed including how the extracts from the leaves and berries may be obtained for use to control insect pests. Finally the future of botanical insecticides in integrated pest management is reviewed.

Chapter 3 provides a general outline on the materials and methods regarding the plant materials collection and extraction. The assays (bioassays and plant assays) that were carried out to evaluate the effects of the plant parts on aphids are also explained in this chapter. A preliminary investigation of three concentrations for each plant part (young leaves, ripe berries and unripe berries) was carried out using bioassays. These were followed up with other bioassays (Chapter 4) and plant assays (Chapter 5) using the most appropriate concentration from the preliminary bioassays. Phytochemical analysis of the leaves and berries was carried out using standard methods based on colour changes and precipitation in reactions.

Chapter 4 is about the detailed bioassays to evaluate the effect of *S. panduriforme* on the cabbage aphid. The effects of plant material to solvent concentration, solvent type and plant part were determined. The effect of aqueous, ethanol, hexane and di-ethyl ether extracts from the three plant parts (leaves in form of leaf powder, fresh unripe berries and ripe berries both fresh and dried) were evaluated. Three concentrations from aqueous and ethanol extracts were evaluated in preliminary leaf dip residual bioassays from which the most effective concentration was evaluated using three bioassay techniques (leaf dip residual bioassays, aphid and leaf dip bioassays and aphid dip bioassays). Choice and no choice bioassays were carried out to determine the effect of aqueous extracts and topical bioassays were also carried out to determine the effect of dried crude solvent assisted extracts.

Chapter 5 provides details of the plant assays using field and screen house plants. The effectiveness of aqueous extracts from the leaves and berries of *S. panduriforme* was evaluated against the cabbage aphid mortality. Pot plants in the screen house were used to evaluate the effectiveness of the extracts on artificially infested populations under simulated conditions and field plants were used to evaluate the effectiveness of the extracts on natural infested aphid populations in the field.

Chapter 6 is on qualitative phytochemical evaluation to examine the presence of four main groups of chemical constituents present in a plant (alkaloids, flavonoids, phenolics and saponins) in aqueous and ethanol extracts from leaves and berries of *S. panduriforme*. These groups are known to have various aphicidal activities.

Chapter 7 is a general summary of the research which summarizes the research chapters. Research findings from the bioassays, plant assays and phytochemical analysis are discussed and some recommendations are suggested. The bioassays and plant assays results provide a body of knowledge on how *Solanum panduriforme* can be used to control aphids on brassicas in areas where it is abundantly growing. The phytochemical evaluation provides bench mark information for standardisation of a commercial botanical insecticide.

CHAPTER 1: INTRODUCTION

1.1 Background

Brassicas such as cabbage, rape (indigenous and exotic types) and kale are important vegetable crops grown for home consumption and market gardening in eastern and southern Africa (Jackson 1997; Maina & Maina 2008). Cabbage is one of the most common vegetables grown for subsistence by almost 80% of the small scale rural farmers in South Africa (Dennill & Pretorius 1995). Rape is a popular vegetable crop in Zimbabwe grown for market gardening and home consumption (Jackson 1997). Kale and cabbage are mainly grown by peri-urban women farmers in Kenya to generate income for family needs (Maina & Maina 2008).

Like any other crops, brassicas are attacked by insect pests, which include aphids, diamond back moth, white grubs, bagrada bugs, cutworms, leaf-miner, cabbage moth, cabbage webworm, cabbage looper and cabbage sawfly (Varela *et al*. 2003). Aphids are considered to be major and important insect pests in the production of most *Brassica* species in Zimbabwe, Malawi, Mozambique, Kenya, Uganda, South Africa and Zambia (Dobson *et al*. 2002; Nyirenda *et al.* 2011; Turner & Chivinge 1999 Varela *et al*. 2003). Aphids feed by sucking sap from plants, causing yellowing and curling of leaves, stunted growth and sometimes death of infested plants. Aphids are vectors of more than 30 virus diseases (Varela *et al.* 2003). They reduce yields and quality of harvested leaves and brassica heads directly through their feeding and indirectly through disease transmission. The damage from the aphids has a negative consequence on food security for rural people and economic instability for peri-urban women who rely on market gardening to generate income to meet basic family needs.

Farmers use synthetic insecticides such as deltamethrin and cypermethrin to control aphids (Nyirenda *et al*. 2011; Turner & Chivinge 1999). According to Buss & Park-Brown (2009), the use of synthetic insecticides results in widespread environmental contamination, toxicity to non-target organisms and most importantly, negative effects on human health. The use of synthetic insecticides by most farmers is reportedly due to lack of sufficient knowledge on the effectiveness of pesticidal plants, with some farmers not even aware of insecticidal plants (Nyirenda *et al.* 2011). Few farmers use insecticidal plant extracts as cheaper and safer alternatives to chemical insecticides (Nyirenda *et al*. 2011; Stevenson *et al.* 2012)

Over 20 different pesticidal plant species have been reported by farmers in Zambia, Malawi and Zimbabwe. *Solanum panduriforme* is among these plants (Nyirenda *et al*. 2011; Stevenson *et al.* 2012). The effectiveness and efficacy of most of the pesticidal plants has not been evaluated (Nyirenda *et al*. 2011; Stevenson *et al.* 2012). Although few farmers in the region use aqueous extracts from the ripe fruits of *S. panduriforme* to control insect pests on vegetables such as rape, cabbages and tomatoes and for veterinary purposes (Masingi *et al.* 2008; Matu 2008; Nyirenda *et al*. 2011; Stevenson 2012), the effectiveness and efficacy of the plant has not been well tested for it to be used as a botanical insecticide by farmers with confidence (Dobson *et al.* 2002; Masingi *et al*. 2008; Mhazo *et al*. 2011; Stevenson *et al.* 2012). There are no reports on standardised extraction methods and long-term toxicity of the ripe berries. Other plant parts of *S. panduriforme* (leaves and unripe berries) have neither been used nor evaluated for their effectiveness. There is no information on effectiveness of the extracts from the leaves and unripe berries. Besides bioactive compounds from aqueous extracts, compounds from other solvents have not been tested for their effectiveness. *Solanum panduriforme* has the ideal properties of an insecticidal plant, according to Silva-Aguayo (2009). It is perennial, widely distributed and has additional medicinal uses such as treating toothache and skin infections (Matu 2008) as well as for and veterinary purposes (Masingi *et al.* 2008; Because of the insecticidal properties, *S. panduriforme* has potential to be developed into a commercial botanical insecticide or for it to be adopted by more farmers especially subsistence farmers who might use it with minimum processing.

The quality and composition of plant extracts depend on the plant part used, the solvent used for extraction, the nature and concentration of the solvent, time of extraction and the extraction procedure or process (Chiffelle *et al.* 2009; EFSA 2009; Mekuaninte *et al.* 2011; Tiwari *et al.* 2011). The toxicity of botanical pesticides also varies within the same species from different locations due to natural plant chemistry; bioactive compounds may be present in different quantities depending on location due to climatic conditions (EFSA 2009; Leatamia 2003). Botanical extracts formulations therefore need to be standardised for them to be effectively used by farmers. According to Isman (2006), the variation in performance of a particular botanical insecticide, even when prepared by the same process, is one of the drawbacks for adoption of botanical insecticides by farmers. Due to the variation on the effectiveness of the botanical insecticides, a certain degree of chemical standardisation based on the active ingredient(s) is required to provide a reliable level of effectiveness and efficacy to the users (Isman 2006; Leatamia 2003).

The future of botanical pesticides for agricultural use has been emphasised in terms of search for flora for bio-pesticidal properties against common agricultural pests, of which over 250 000 plants have been identified worldwide (Farnsworth 1990; Silva-Aguayo 2009). Only a few plants have been properly evaluated for their use as pesticidal plants (Silva-Aguayo 2007; Farnsworth 1990). Data on effectiveness and long-term toxicity is unavailable for most plants, and tolerances for residues on food crops have not been established (Buss & Park-Brown 2009, Dobson *et al*. 2002). Isolation, identification and evaluation of the active components and synthesis of the plant components for commercial use (Prakash *et al*. 2008; Silva Aguayo 2009) are crucial for the pesticidal plants to be used in integrated pest management strategies.

Researchers have suggested that botanical extracts from locally available insecticidal plants be made available to farmers for use together with the information on their effectiveness and efficacy (Iqbal *et al.* 2011; Wabale & Kharde 2010). More specialised studies on scientific formulations and use of plant-based pesticides to ensure their safety and effectiveness for food security and environment protection have also been recommended. According to Chowanski *et al*. (2016) and Mugisha-Kamatenesi *et al.* (2008), recommendations from specialised studies provides valuable information for further research, extension work and industrial accomplishments of commercial botanical insecticides production.

It has been argued that the greatest benefits from botanical plants might be achieved in developing countries, where pesticide poisoning in humans is most prevalent according to Isman (2006); botanicals are assumed to be less dangerous than the widely used synthetic insecticides. Research carried out in Africa suggests that extracts of locally available plants can be effective as crop protectants, either used alone or in mixtures with conventional insecticides at reduced rates (Isman 2006; Khalequzzaman & Nahar 2008). Successful control of brassica pests, particularly aphids and diamondback moth, has already been shown with neem products alone or in combination with other pesticides (Khalequzzaman & Nahar 2008; Varela *et al*. 2003). Achievements on effective use of pesticidal plants based on pyrethrum, neem and rotenone have also been made, resulting in production of more refined products for insect control (Isman 2006; Sallam *et al*. 2009). It may also be possible to develop a *Solanum panduriforme* based botanical insecticide which can be available for use by farmers to control aphids on vegetables especially brassicas.

1.2 The research problem

Aphids are considered serious and important insects that attack important vegetable crops for home consumption and income generation in southern Africa and worldwide (Kessing & Mau 1991; Nyirenda *et al*. 2011; Prakash *et al*. 2008; Turner & Chivinge 1999). The aphids cause damage directly through their feeding on leaves and indirectly as vectors of virus diseases, resulting in quality and quantity losses. The predominant use of synthetic insecticides by farmers to control aphids is associated with environmental, economic and human health problems (Buss & Park-Brown 2009; Dobson *et al.* 2002; Isman 2006). Botanical insecticides have the potential to safely and cheaply control insect pests, serving as alternatives to dangerous and expensive chemical insecticides. The effectiveness and efficacy of botanical plants like *Solanum panduriforme* need to be ascertained so that more farmers may adopt their use to control problem insect pests.

1.3 Justification

The use of synthetic insecticides in crop protection programmes around the world has resulted in adverse effects on the environment, pest resurgences and insecticide resistance problems (Dobson *et al*. 2002; Prakash *et al*. 2008). The synthetic insecticides also have lethal effects on non-target organisms in agro-ecosystems and direct toxic effects on the people who use the insecticides and the consumers of treated products (Buss & Park-Brown 2009; Prakash *et al.* 2008). Botanical insecticides are believed to have environmental, economic and health advantages over synthetic insecticides. They are assumed to be less expensive and easily available because of their natural occurrence (Isman 2006; Wabale & Kharde 2010). However, only a few plant species have been evaluated for use as insecticidal plants. *Solanum panduriforme* fresh ripe berries are sometimes used by few farmers to control problem insects on vegetables and livestock but there is little evidence to support its effectiveness (Masingi *et al.* 2008; Stevenson *et al.* 2012). There is dearth of information on effectiveness of the leaves and unripe berries in different forms (fresh and dried), besides a little information on aqueous fresh ripe berries. This information is crucial for the plant extracts to be adopted for use by more farmers or for it to be synthesised into a commercial botanical insecticide by industry.

1.4 Research questions

1. Do the extracts from (1) dried young leaves, (2) dried ripe berries, (3) fresh ripe berries and (4) fresh unripe berries of *Solanum panduriforme* cause mortality of the cabbage aphid, *Brevicoryne brassicae*, on brassicas?
2. Is the bioactivity of extracts from *S. panduriforme* affected by the solvent used for extraction?
3. Do the aqueous and ethanol extracts from the young leaves, fresh ripe berries and fresh unripe berries of *S. panduriforme* contain the same bioactive compounds?

1.5 Aim and objectives

The aim of this research was to evaluate effectiveness of extracts from *Solanum panduriforme* leaves and berries on the mortality of the cabbage aphid, *Brevicoryne brassicae,* whose host range is restricted to brassicas only.

The main objectives were:

1. To investigate the effects of aqueous extracts, ethanol extracts and solvent assisted (hexane and diethyl ether) extracts of S*. panduriforme* on aphids (*Brevicoryne brassicae)* mortality using laboratory bioassays.
2. To determine the effectiveness of aqueous extracts from *S. panduriforme* on cabbage aphids *(Brevicoryne brassicae)* mortality on brassica plants using screen-house and field plant assays.
3. To identify the bioactive compounds from aqueous and ethanol extracts of *S. panduriforme* using qualitative phytochemical analysis.

1.6 Hypotheses

1.6.1 Null hypothesis

There is no difference in mortality of the cabbage aphid, *Brevicoryne brassicae,* treated with aqueous extracts, ethanol extracts and solvent assisted (hexane and di-ethyl ether) extracts from dried leaves, dried ripe berries, fresh ripe berries and fresh unripe berries of *Solanum panduriforme*; the extracts contain the same bioactive compounds.

1.6.2 Alternate hypothesis

There is a difference in mortality of the cabbage aphid, *Brevicoryne brassicae,* treated with aqueous extracts, ethanol extracts and solvent assisted (hexane and di-ethyl ether) extracts from dried leaves, dried ripe berries, fresh ripe berries and fresh unripe berries of *Solanum panduriforme;* the extracts do not contain the same bioactive compounds.

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CHAPTER 2: LITERATURE REVIEW

2.1 Importance of brassicas

Vegetables are an important source of fresh food for households, forming an important part of the diet in most households in Africa (Dobson *et al.* 2002; Maina & Maina 2008). They play a major role, together with fruits, in supplying essential minerals, vitamins and fibre. Brassicas, sometimes referred to as crucifers or the cabbage family, constitute the majority of cultivated Brassicaceae (formerly Cruciferae) in eastern and southern Africa (Maina & Maina 2008; Varela *et al*. 2003). The main brassicas grown in the eastern and southern Africa include cabbage (*Brassica oleracea* L. var. *capitata*), kale or choumolea (*B. oleracea* L. var*. acephala*), chinese cabbage (*B. campestris* L. var. *chinensis, B. campestris campestris* L. var. *pekinensis*), cauliflower (*B. oleracea* L. var. *botrytis*) and rape (*B*. *carinata* and *B. juncea* the indigenous types and *B. napus*, the exotic type). Other brassicas grown in the region are broccoli (*B. oleracea* L. var. *italica*), Brussels sprouts (*B. oleracea* L. var. *gemmifera*), kohlrabi (*B. oleracea* L. var. *gongylodes* L.), savoy (*B. oleracea* L. var. *sabauda*), swede (*B*. *napus* L. var. *napobrassica*), turnip (*Brassica rapa L*. var. *rapa*), radish (*Raphanus sativus* L. var *hortensis*) and horseradish (*Armoracia rusticana* Gaertn.). In southern Africa, the most frequently grown brassicas are cabbage (*B. oleracea* var *capitata*), rape (*B. napus*), Chinese cabbage *(B. campestris* var *chinensis*) and mustard rape (*B. juncea*) (Dobson *et al*. 2002; Nyirenda *et al*. 2011). The brassicas are grown mainly for domestic use and for the local market as valuable sources of vitamins and minerals and cash for small-scale farmers in rural and peri-urban areas (FAO 2009; Maina & Maina 2008; Varela *et al.* 2003). They are cultivated, mostly in small back or front yard gardens and also in medium to large-scale commercial enterprises (FAO 2009; Maina & Maina 2008).

Cabbage is one of the most popular vegetables in South Africa and Lesotho (DAFF 2012; Phoofolo *et al*. 2013). It is an important subsistence crop in South Africa and it is estimated that 80% of small-scale rural farmers who have access to water grow cabbage. Although it is grown country-wide in South Africa, production is more concentrated in Mpumalanga and the Camperdown and Greytown Districts of KwaZulu-Natal (DAFF 2012). In East Africa, cabbage is widely consumed by high income families more than low income families (Maina & Maina 2008). In Kenya, rural women are more involved than men in the farming and marketing of brassicas, as they secure a source of continuous income to support the family needs and to enable small

farms to remain financially viable, especially in the rapid growing peri-urban farming sector (Ochieng *et al.* 2010). Kale (*B. oleracea* L. var*. acephala)* is the most consumed green vegetable in both urban and rural areas of East Africa (Maina & Maina 2008). Rape (*B. napus)* is popularly grown for home consumption and market gardening in Zimbabwe by both rural farmers and peri-urban farmers (Dobson *et al*. 2002; Jackson 1997).

Vegetables in the cabbage family contain protective phytochemicals called glucosinolates that help the body eliminate carcinogens (Woverton 2011). The phytochemicals work as powerful antioxidants and help to protect against breast, colon and prostate cancer. Cabbage is a good source of thiamin, calcium, iron, magnesium, phosphorus and potassium, and a very good source of dietary fibre, vitamin C, vitamin K, vitamin B6, vitamin B1 (thiamin), vitamin B2 (riboflavin) and the minerals folate, calcium and manganese (Maina & Maina 2008). It is very low in saturated fats and cholesterol; a half cup serving of shredded, raw cabbage contains 8.3 calories and only 0.1 g of fat, making it an ideal food for low-calorie and low-fat diets. Cabbage has little protein but it serves as a good source of vitamin C, providing 29.8 % of the daily recommended intake per portion (Woverton 2011). It contains high levels of fibre, half a cup serving has 0.8 g fibre, which may contribute to rectal gas and flatulence. Rape and kale are a good source of vitamins (A, B6 and C), minerals (manganese, copper, calcium and potassium) and dietary fibre which are important components in the diet (Maina & Maina 2008). According to Toxopeus & Mvere (2004), the nutritional composition of rape per 100 g edible portion (part of midribs removed) is: water 88.2 g, energy 155 kJ (37 kcal), protein 3.8 g, fat 0.3 g, carbohydrate 4.8 g, calcium 250 mg, magnesium 85 mg, phosphorus 81 mg, iron 1.7 mg, carotene 1990 µg, thiamin 0.07 mg, riboflavin 0.06 mg, niacin 0.8 mg, ascorbic acid 55 mg. Like other members of brassicas, rape contains high levels of glucosinolates, which have antioxidant and anticancer activities. Compared to most leafy vegetables, the content of micronutrients in rape is high and the iron is in an easily digestible form (Toxopeus & Mvere 2004).

The adaptability of cabbage and the lower production costs of rape and kale make these brassicas more popular and economic for production by farmers. Cabbage is a popular vegetable throughout the world due to its adaptability to a wide range of climatic conditions and soils. It is relatively easy to produce and store (DAFF 2012; Maina & Maina 2008). Cabbage can be grown throughout the year when optimum temperatures are 18 °C – 20 °C. Well drained moisture retentive loamy soils well supplied with organic matter are best for cabbage production which can be transplanted from seedlings or directly seeded (DAFF 2012). Rape grows best

under cool conditions with optimum temperature of 15 °C – 20 °C. It is grown exclusively by seed in well drained fertile soils with high organic matter (Toxopeus & Mvere 2004). Mustard rape and oilseed rape is always direct seeded. Kale is much easier to produce compared to other vegetables, it requires fewer chemical inputs and labour; when sufficiently nourished with compost manure and well-watered, it produces huge volumes of leaves, which can be harvested repeatedly several times a week from the same plant (Maina & Maina 2008). This makes it to be readily and cheaply available to consumers. The lower production costs of kale and rape translate into lower selling prices in the market, making it affordable to households with less income.

2.2 Aphids as major insect pests of brassicas

The problems from insect pests of brassicas in eastern and southern Africa were identified during a planning workshop held in Malawi with participants from seven countries (Kenya, Malawi, Mozambique, South Africa, Uganda, Zambia and Zimbabwe) and they were revised during a second meeting held in Kenya (Varela *et al*. 2003). The main insect pests were identified as aphids, diamondback moth (DBM), head-borer *Hellula* spp. and bagrada bugs. Other identified insect pests that attack brassicas are white grubs, cutworms, leaf-miner, cabbage moth, thrips, cabbage webworm, cabbage looper and cabbage sawfly.

Aphids are considered major insect pests that attack brassicas in Zimbabwe, Malawi, Zambia and South Africa (Nyirenda *et al*. 2011; Turner & Chivinge 1999) and in India (Prakash *et al*. 2008). Three different species of aphids are of importance on brassicas in eastern and southern Africa (Varela *et al*. 2003; Dobson *et al*. 2002). These are *Brevicoryne brassicae* (the mealy cabbage aphid or the grey cabbage aphid), *Lipaphis erysimi* (the false cabbage aphid also known as the turnip aphid *Lipaphis pseudobrassicae*) and *Myzus persicae* (the green peach aphid). The mealy cabbage aphid is restricted to brassicas only and not any other plants outside the family. It is the most common species that attacks cabbages, cauliflower, rape, broccoli, Brussel sprouts, turnips and mustards (Kessing & Mau 1991). It is a more damaging insect pest of cabbage, cauliflower and Brussel sprouts than on other brassicas (Mau & Kessing 1991). The false cabbage aphid (*L. erysimi)* is a major pest of brassicas throughout the world including eastern and southern Africa and a minor pest on other important crops such as beans, beet, spinach, pea, celery, onion, soybean, cucumber and potatoes (Dobson *et al*. 2002; Kessing & Mau 1991; Varela *et al.* 2003).The green peach aphid (*M. persicae*) has a wide range of host plants, including many economically important crops such as peach, potato and tomato; it also

attacks many ornamental crops such as carnations, chrysanthemums, poinsettias and roses (Mau & Kessing 1991; Varela *et al.* 2003). It is considered a very important cabbage insect pest but is more abundant in temperate regions than in the tropics (Mau & Kessing 1991; Varela *et al.* 2003). The mealy cabbage aphid (*B. brassicae*) and the green peach aphid (*M. persicae*) are considered the most common species found on cabbages (DAFF 2012; Dobson *et al*. 2002).

2.3 Biology and ecology of aphids

The aphid species that attack brassicas occur in colonies mostly on the underside of leaves. The colonies are made up of mixed winged or wingless adults and nymphs and occasionally mixed aphid species on one plant (Varela *et al.* 2003; personal observations). Wingless adult forms are the most prevalent. Established colonies are made up of several generations that are sedentary on the same plant. Aphid species generally have similar life cycles which can be simple or complicated (Nayar *et al.* 1990; Varela *et al*. 2003.) Females can reproduce with or without mating; they may lay eggs or give birth to wingless offspring or immature young aphids known as nymphs. Eggs are very tiny, shiny-black, and are found in the crevices of bud, stems, and bark of woody plants (Nayar *et al.* 1990). Nymphs look like the wingless adults but are smaller and usually lighter in colour. For most species nymphs grow quickly in warm weather becoming adults and start to reproduce in about 7 to 10 days (Mau & Kessing 1991; Nayar *et al.* 1990; Varela *et al.* 2003).

In the warm parts of the world, as in the tropics, reproduction is asexual, no male aphids are produced; the female aphids do not lay eggs but give birth to nymphs (Mau & Kessing 1991). A female can produce from 20 to over 100 nymphs (Nayar *et al.* 1990; Varela *et al.* 2003). In southern Africa, the life cycle of aphids is simple; female aphids are parthenogenic and viviparous. Nymphs are produced continually, and as the colonies become too dense, winged females are formed which migrate to other plants to start other colonies (Mau & Kessing 1991; Nayar *et al.* 1990; Taylor 2012). Warm and dry weather is particularly favourable for the rapid increase of aphid populations. In southern Africa, aphids are generally most prevalent during warm, dry weather, when brassicas are produced with irrigation and suppressed during the rainy season, if the rains are frequent and heavy (Taylor 2012).

The adults of the mealy cabbage aphid (*B. brassicae*) measure between 1.6 and 2.8 mm in length (Taylor 2012; Varela *et al*. 2003). They are greyish-green or dull mid-green in colour and are covered with a fine waxy grey mealy powder (Kessing & Mau 1991; Varela *et al*. 2003).

Survival is least at temperatures above 30 °C. Mortality is lowest at 20 °C, which is also the optimum temperature for its development (Varela *et al.* 2003). *Brevicoryne brassicae* is a cold season aphid in southern Africa, with heavy infestations in winter than in spring and summer; in east Africa it is particularly serious during dry months of the year (Varela *et al.* 2003).

The adults of the false cabbage aphid (*L. erysimi)* are between 1.4 and 2.4 mm long (Varela *et al.* 2003; Sanderson 2012). Wingless aphids are yellowish green, pale green, grey green or olive green in colour, and can be distinguished from the cabbage aphid because they are not covered or only slightly covered by wax. Winged aphids have a dusky green abdomen with conspicuous dark lateral sclerites, and dusky wing veins. Temperature is an important factor in the seasonality of the false cabbage aphid; extremely high numbers are found during the warm season, becoming scarce in the cool season (Varela *et al.* 2003). Heavy rainfall adversely affects its reproduction and development.

The adults of the green peach aphid (*M. persicae*) are between 1.2 and 2.3 mm long. The nymphs are greenish turning yellowish as they grow and closely resemble adults. Nymphs that develop into winged adults may be pinkish (Mau & Kessing 2009). The adult wingless form varies considerably from yellow, through all shades of green, to pink, red and almost black. The winged form has a black central abdominal patch on the upper surface, but a pale underside (Mau & Kessing 1991). Wingless forms are usually uniformly green in colour, with a darker thorax. Antennae lengths are two-thirds of the body and the cornicles are fairly long. The life cycle varies considerably, depending on cold temperatures (Nayar *et al*. 1990; Varela *et al.* 2003). Twenty to 30 generations have been reported per year (Mau & Kessing 1991), an indication of how aphids' populations can rapidly increase causing damage to host crops.

For botanical insecticides to effectively control aphids, it is important to monitor aphid populations to avoid build up to economic thresholds. It has been reported that botanical insecticides can be used to effectively control aphid populations with frequent repeated spraying regimes (Bhat & Yubak-Dhoj 2005). Because botanical insecticides are assumed to be safer and cheaper, frequent sprays may not be dangerous to the environment, non-target organisms, the insecticide user and the consumer. Knowledge of the biology and ecology of the aphids helps with timely scouting to enable frequent applications that can greatly improve botanical insecticides effectiveness. Aphids generally have short life cycles and therefore populations can be managed with frequent applications targeting mostly the nymphs which are much more

susceptible than adult aphids (Atteyat *et al*. 2012). The sedentary nature of aphid colonies makes it possible to use spot sprays on affected plants or plant parts using botanical insecticides resulting in aphids control and elimination of disease transmission.

2.4 The effects of aphids on brassicas

Aphids have long, piercing mouth parts which allow them to feed by sucking sap or juices from the plant. The different species generally cause similar damage on the brassica plants (Kessing & Mau 1991; Sanderson 2012; Taylor 2012; Varela *et al.* 2003). Damage by aphids is through direct feeding and indirectly through virus diseases transmission. In eastern and southern Africa the *B. brassicae*and the *L. erysimi* are important vectors of diseases such as cabbage black ring spot, cabbage ring necrosis and mosaic diseases of cauliflower, radish and turnip. The green peach aphid transmits more than 100 viruses in over 40 different plant families including brassicas, beans, sugarcane, potato, citrus and tobacco. *Brevicoryne brassicae* is a vector of more than 30 viruses; the most important are cauliflower mosaic virus and turnip mosaic virus (Dobson *et al*. 2002; Kessing & Mau 1991; Varela *et al.* 2003). Aphid-transmitted viruses often result in deformed and stunted plants.

The direct feeding by aphid colonies causes leaf curl, discolouration, stunted growth and even the death of infested plants. If in large numbers, aphids remove sufficient sap to kill the leaves and the growing tip (Taylor 2012; Varela *et al.* 2003). The feeding on expanding leaves causes twisting and discolouration of the leaves. In heavy infestations, the aphids excrete copious amounts of plant sugars from the sap as honeydew, on which unpleasant black sooty mould grows. Heavy coating with honeydew and sooty moulds may reduce photosynthesis, thus affecting plant growth and yield; this affects and reduces the quality and quantity of the crop (Kessing & Mau 1991; Sanderson 2012; Taylor 2012; Varela *et al.* 2003)

The cabbage aphid prefers the youngest tissue and highest portions of the plant. Cabbage is more sensitive to aphid damage before heading due to possible distortion of the heads (Kessing & Mau 1991; Varela *et al.* 2003). When the aphids hide within the heads they can be a serious contaminant of marketed heads of broccoli and cabbages. Aphids become a major problem on rape plants when the plants develop from seedling stage; this is when the crop is more sensitive to the aphids (Toxopeus & Mvere 2004) and thus requires effective safe control methods which may include use of botanical insecticides.

2.5 Control of aphids

The control options for aphids on brassicas include natural (biological), cultural, botanical and chemical control (Dobson *et al*. 2002; Mahr *et al.* 1993; Varela *et al* 2003). Natural control is through parasitic wasps, predators and entomophagous fungi. Cultural control is through practices like destruction and removal of infested crop residues to prevent infestation of adjacent crops, crop rotations and intercropping and use of resistant varieties. Botanical control is through the use of bioactive components from pesticidal plants and chemical control is through the use of synthetic chemicals. When these methods are combined to control aphids it is called integrated pest management; however the control methods may interfere with each other (Dobson *et al.* 2002). Integrated pest management on brassica crop production is not much because the use of pesticidal plants, cultural practices and resistant varieties constitutes a smaller portion of the pest control options (Varela *et al.* (2003), the majority of farmers use synthetic insecticides only to control problem insect pests. It is important to use the aphid control options in a way that will not interfere with the effectiveness of another option. Use of botanical insecticides and synthetic insecticides to control aphids should not interfere with natural control whereby the natural enemies are also killed.

Botanical insecticides have been found to be relatively safer than synthetic insecticides in protecting the natural enemies (Amoabeng *et al.* 2013; Dadang *et al*. 2009). When diamondback moth and aphids were controlled using botanicals and entomophagous fungi, substantial numbers and species of natural enemies were observed in untreated plots and in plots that were sprayed with entomopathogenic fungi and neem based botanical extracts when compared to those sprayed with synthetic insecticides (Waiganjo *et al*. 2008), an indication that botanical insecticides are compatible with natural control. The natural enemy densities from botanical extract treated plants were similar to the water control; but aphid infestation in water control plots was high with lower yields than botanical extracts treated plots an indication that botanicals are effective against aphids (Waiganjo *et al*. 2008).

When botanical insecticides were used to control diamond back moth and the cabbage aphids, less toxic effects were observed on natural enemies, ladybirds, hoverflies and spiders than the conventional insecticide (Amoabeng *et al.* 2013). Another study indicated that aqueous *Melia azedarach* leaf extracts can have a significantly negative impact on first-instar larvae of *Plutella xylostella* with no direct negative impact on their parasitoids *Cotesia plutellae* and *Diadromus*

*collaris (*Charleston *et al*. 2001). The results of a study on the effect of botanicals on natural enemies of pistachio psyllid showed that Tondexir commercial botanical insecticide (pepper extract) has less effects on two natural enemies than two chemical insecticides acetamiprid and hexaflumuron (Abbad & Besheli 2013). It was recommended that Tondexir should be used to control the pistachio psyllid. This becomes more advantageous for organic farmers and organic consumers. The commercial flavonoids from citrus fruits had a very slight impact on the parasitoid of the woolly aphids when compared to the effects of imadacloprid a synthetic insecticide (Atteyat *et al* 2012). However some synthetic insecticides like acephate and pirimicarb have been reported to be relatively safe to natural enemies (Taylor 2012). These may be used together with botanical insecticides in integrated pest management approaches. Some studies have, however, indicated that higher concentrations of botanicals may have detrimental effects on natural enemy populations (Bugg *et al.* 2008). It is therefore important to use appropriate well researched botanical insecticides concentrations to control target insect pests.

2.5.1 Natural control

Aphids are naturally controlled by parasitic wasps, predators and entomogenous fungi. Some of the natural enemies are already in the field before the aphids attack the crop while others come in as aphid colonies build up in response to the presence of aphids (Bugg *et al.* 2008; Mahr *et al.* 1993). The natural enemies can reduce aphid populations or may totally eradicate the aphids from the crop (Bugg *et al*. 2008). It is therefore important to protect these natural enemies. Botanicals have been found to have less toxic effects on non-target organisms and are assumed to be safe for use against natural enemies (Buss & Park-Brown 2009; Silva-Aguayo 2009; Prakash *et al.* 2008).

Parasitic wasps or parasitoids from the families Aphidiidae and Aphelinidae include the parasitic wasps *Aphidius colemani* and *Aphidius ervi*. The most important parasitoid of the cabbage aphid and the false cabbage aphid is the braconid wasp, *Diaeretiella rapae* (Mahr *et al.* 1993; Varela *et al.* 2003). Several species of parasitic wasps oviposit on aphid nymphs and develop in them resulting in parasitized aphids known as mummies (Bugg *et al*. 2008). The mummies can easily be recognised as they turn brown, become hardened and remain stuck to the plant surface. Farmers may use the safe botanical insecticides in integrated pest management (IPM) approaches which are natural enemy friendly to enhance the activities and reproduction of

parasitic wasps that result in high numbers of mummified aphids leading to decreased aphid populations.

The most important aphid predators are predatory bugs (Anthocoridae, Miridae, Nabidae), carabid beetles (Carabidae), soldier beetles (Cantharidae), predatory gall midges (Cecidomyiidae), lacewings (Chrysopidae), ladybird beetles (Coccinellidae) and hoverflies (Syrphidae) (Bugg *et al*. 2008; Mahr *et al.* 1993; Varela *et al*. 2003). The relative importance of predators varies with location and season. Some predators like lady birds are seldom seen during the cold season, thus aphid populations rapidly build up in the absence of natural control especially during this time of the year when the conditions are conducive for their optimum growth and development; other means of control using botanical extracts become valuable. Other predators like the syrphid larvae may be attacked by other parasitic wasps (Bugg *et al.* 2003). Many of the predators need nectar and pollen to feed on which may not be available within the field or crop of concern. It may be necessary to grow insectary crops around the fields which provide flowers for nectar and pollen. This encourages natural enemy population build up for integrated pest management approaches.

Depending on climatic conditions and crops, fungi that cause diseases of insect pests (entomopathogenic fungi) can contribute to a rapid decline of aphid populations. According to Mahr *et al.* (1993), entomogenous fungal epidemics can cause significant cabbage aphid populations decline. Fungi that attack aphids include *Pandora neoaphidus, Beauveria bassiana, Zoopthora phalloides, Canidiobolus obscures* and *Entomophthora plancchoniana* (Bugg *et al*. 2008). The fungi that attack aphids are important to consider in integrated pest management when humidity is high, but since most brassicas are produced during winter when humidity is low they may not be effective in reducing cabbage aphid populations. It is important to distinguish the entomogenous fungi from the sooty mould fungi to ensure that there is aphid control from fungi (Bugg *et al*. 2008). The entomogenous fungi appear as whitish to pink growths whereas the sooty mould fungus is dark.

2.5.2 Use of synthetic insecticides

Many organophosphate insecticides of varying toxicity can be used to control aphids on different brassicas. These include demeton-s-methyl, diazinon, dichlorvos, malathion, methamidophos and mevinphos (Kessing & Mau 1991; Taylor 2012). Malathion, diazinon and dementon-s methyl, acephate, alpha-cyperrnethrin, bifenthrin, buprofezin, chinomethionat, fenitrothion,

fenvalerate, imidacloprid, permethrin, pirimicarb, pymetrozine and trichlorfon are commonly used insecticides in east and southern Africa (Varela *et al.* 2003).

The use of synthetic insecticides in crop protection programmes around the world has resulted in insect resistance to some insecticides and insect resurgence together with lethal effects on non-targets and direct toxicity to users of the chemicals and sprayed products (Dobson *et al*. 2002; Prakash *et al*. 2008). Pyrethroids such as cypermethrin, deltamethrin and lambdacyhalothrin have given effective control of aphids in east and southern Africa (Varela *et al*. 2003) but resistance to the various insecticides (carbamate and organophosphate such as cabaryl and malathion, and/or pyrethroid such as cypermethrin) is common in aphids including the cabbage aphid *B. brassicae* and the lettuce aphid, *Nasonovia ribisnigri* (Workman *et al.* 2004). Aphids and lepidoptera larvae resistance to methamidophos, profenofos, chlorpyrifos, endosulfan, cypermethrin and deltamethrin has been detected across the world in Africa, Asia, Europe, USA and New Zealand (Moslemi *et al*. 2011; Munir & Muhammad 2005; Sanderson 2012; Townsend 2012; Workman *et al.* 2004). When aphid populations develop resistance to certain chemicals, the chemicals become ineffective when used to control the aphids.

Synthetic insecticides have been reported to be dangerous to non-target insects, the users of the insecticide, the consumers of sprayed products and the environment in many ways either through misuse or excessive use (Dobson *et al*. 2012). Insecticide residues in many agricultural products are a main concern by consumers especially the ethical organic product consumers who insist on organically produced products. Synthetic insecticides are expensive, and many resource poor farmers may not afford them (Dobson *et al*. 2002) when compared to botanicals which can be used at no cost or minimal cost; farmers can collect the plants from the wild and prepare the extracts for use using water as a cheaper readily available solvent (Amoabeng *et al.* 2013). Many small-scale and subsistence farmers in developing countries also do not have the resources to buy and apply the dangerous chemical pesticides (Charleston *et al*. 2001).

Most subsistence farmers in southern Africa predominantly use expensive dangerous synthetic insecticides to control aphids (Nyirenda *et al*. 2011; Obopile *et al*. 2008; Sibanda *et al*. 2000; Turner & Chivinge 1999). Sometimes the farmers use the wrong synthetic insecticides because the right insecticide is not available or affordable at the time the farmer wants to spray his crop (Dobson *et al*. 2002). The reasons for using synthetic insecticides over safer botanical

insecticides according to Stevenson (2010) and Nyirenda *et al*. (2011) are that farmers are accustomed to synthetic insecticides which make them the only available option.

2.5.3 Botanical insecticides

Botanicals insecticides are toxins that are derived or extracted from secondary plant metabolites (Chowański *et al.* 2016; Singh & Chauhan 2014). They are naturally occurring toxins used by plants to repel insects, to make a plant unpalatable or to attract insects to poison them. Several authors have used different names such as natural insecticides, bio-insecticides or bio-rational insecticides (Asogwa *et al*. 2009; Buss & Park-Brown 2009; Chowański *et al*. 2016; Sallam *et al*. 2009). As mentioned earlier the use of botanical extracts especially when alternated with synthetic insecticides may help to reduce costs to farmers, prevent insecticide resistance, protect the natural enemies and most importantly achieve effective insect pest control (Dadang *et al*. 2009). Secondary plant metabolites can disturb insect development, lead to malformations or malfunctions, extend the duration of developmental stages or act as repellents (Bandeira *et al*. 2013; Golawaska *et al.* 2008a; Golawaska *et al.* 2008b; Habimana & Hakizayezu 2014; Ntonifor *et al*. 2010; Tennyson *et al*. 2011). This may result in reduced reproduction in the affected populations or cause the migration of the insects away from plants sprayed with these compounds leading to reduced target insect pest populations. The secondary compounds become a good option in integrated pest management approaches.

Effective control of brassica pests, particularly aphids, diamondback moth and other caterpillars has been demonstrated with neem products alone or in combination with other pesticides in east Africa (Varela *et al.* 2003). Neem oil and neem seed extracts have reportedly given effective control of the cabbage aphid, green peach aphid and the false cabbage aphid on brassicas (Varela *et al.* 2003). Neem extracts were reported to be more highly toxic to aphids than carbosulfan, imidacloprid and cypermethrin in a toxicity assay study by Khalequzzaman & Nahar (2008), an additional advantage over the environmental, economic and health advantages of botanical insecticides. Neem-based pesticides deter feeding in many insects such as aphids, thrips, leaf-miner (Lajeunesse 2001; Varela *et al* 2003), this is particularly important in the case of vectors of virus diseases which include aphids and whiteflies.

Neem extracts effectiveness may be reduced in cooler climates making them ineffective on some aphid species including the cabbage aphid which is a cold season aphid with higher populations in winter (Lajeunesse 2001; Varela *et al.* 2003). Effectiveness of botanical extracts

also depends on location where the botanical plants are growing, the location contributes to the chemistry of bioactive components or the presence or absence of some compounds from the plant. Variations in effectiveness of botanical extracts as reported earlier is one of the biggest problems for their adoption by farmers; it is important to research and document how local plants may be used by farmers to control insect pests with confidence. Research results have shown that repeated applications of known botanical insecticidal plant extracts help to achieve effective control (Lajeunesse 2001).

Extracts from garlic and green chilli are effective against aphid infestations on many crops like cabbages, rape and tomatoes (Habimana & Hakizayezu 2014). Other botanicals that are effective against brassica insects include *Lippia* leaf extracts, *Mentha* leaf extracts, *Melea* seed extracts, *Piper* and *Aframomum* spice extracts, *Mutingia* extracts and *Lantana camara* leaf extracts (Baidoo & Adam 2012; Bandeira *et al.* 2013; Gonzalez *et al*. 2011; Mekuaninte *et al.* 2011; Ntonifor *et al*. 2010). Aqueous eextracts from, tobacco leaves, combined garlic and chilli including *S. panduriforme* are effective against the cabbage aphid (Katsvanga *et al*. 2006; Mhazo *et al*. 2011; Stevenson 2010). Generally alcohol extracts are more effective than aqueous extracts but Mekuaninte *et al.* (2011) studies indicated that aqueous extracts may be more effective; significantly higher cabbage aphid mortalitly was obtained from aqueous than methanol extracts of the leaves and seeds of *Melia azadarach* and *Mentha piperita* leaves. Essential oils from *Foeniculum vulgare*, *Pimpinella anisum*, *Rosmarinus officinalis*, *Juglans regia* and *Laurus nobilis* assessed under laboratory conditions for their effect on *B. brassicae* significantly reduced the reproduction potential of the cabbage aphid and resulted in higher mortality (Mustafa & Gazi 2009). The essential oils are also considered an important aphicide.

A survey revealed that some farmers spray their vegetables with aqueous extracts from ripe berries of *S. panduriforme* to control aphids but without evidence to support the insecticidal activity of the plant (Stevenson *et al.* 2012). Aqueous extracts from *S. panduriforme* ripe berries resulted in variable mortality of 60% to 100% under field and pot conditions. This indicates the importance of evaluation of the insecticidal properties of *S. panduriforme* for effective use against the cabbage aphids. Surveys in Malawi and Zambia also indicated that most farmers are aware of at least one pesticidal plant which include *S. panduriforme* but less than half in Malawi and 20% in Zambia use them to control insect pests. The few rural poor farmers who use pesticidal plants use them as a cheaper alternative to chemical pesticides. Use of pesticidal plants by farmers in Zimbabwe revealed that a smaller proportion of vegetable farmers knew

and actually used plant pesticides to control aphids in the production of brassicas (Stevenson 2010), yet they are important in integrated pest management strategies in the region (Dobson *et al.* 2006 ; Varela *et al.* 2003). In east Africa some resource poor farmers use plant based insecticides for crop protection on crops like cocoa, okra and garden egg (Coulibaly *et al.* 2002; Ibekwe *et al*. 2014; Stevenson *et al.* 2012) as a cheaper alternative. However some farmers have reported that pesticidal plants are less effective, resulting in a noticeable loss of crop quality and quantity (Nyirenda *et al*. 2011; Stevenson, 2010). However it has been reported that botanicals are as effective as synthetic insecticides on cowpeas insect pests; tobacco leaf extract at 1% is equally effective as monocrotophos against aphids and *Tephrosia vogelii* is as effective as decis against pod borers (Adebayo *et al.* 2007; Mugisha-Kamatenesi *et al.* 2008).

Regardless of some plants having insecticidal activities, they may be ineffective when farmers use ineffective application rates and inappropriate extraction methods; their indigenous knowledge may be limited in terms of consistent rates and extraction methods for effective control of target insect pests. Some rural poor farmers have shown lack of knowledge on use of the pesticidal plants though they are aware of their existence (Stevenson 2010; Nyirenda *et al*. 2011). As with synthetic insecticides proper rates, use of surfactants (simple household liquid dishwasher) to enable contact and well-adjusted spraying equipment for thorough coverage, contribute to effectiveness of botanical insecticides (Dobson *et al.* 2002). Well documented labelling from well researched information on the use and safety of botanical insecticides is important for successful and effective use by farmers in the form of pamphlets for extension use. Plant materials may be made available to other farmers who may not have easy access to the plants; for the plant materials to be effectively used they should be well labelled in terms of preparation and application. Farmers do not have adequate information on how to prepare botanicals extracts, some even have a feeling that botanicals are as poisonous as synthetic insecticides yet according to Buss & Park-Brown (2009) they are relatively safe due to the fact that they easily degrade on plant leaves and in the soil. According to Stevenson *et al.* (2012) some work has been done on the use of pesticidal plants in Africa but there are very few cheaper commercially available botanical insecticides for use by resource poor farmers. Varela *et al.* (2003) also mentioned that pesticidal plants are used less as a pest control option on brassica crop production.

When compared to synthetic insecticides, botanicals are usually always cheaply available from the wild and affordable, they have short residual period and resistance is unlikely to occur from

botanical insecticides due to their quick degradation (Buss & Park Brown 2009). Besides extraction and preparation costs the cost benefit ratio (CBR) of botanical insecticides over synthetic insecticides is also related to environmental risks from synthetic insecticides which kill beneficial insects (Aziz *et al*. 2013). When neem extracts were used to control the English grain aphid on wheat they had lower cost benefit ratios and more economical than imidacloprid, a neonicotinoid synthetic insecticide, (Aziz *et al*. 2013). The application of neem seed kernel extract (NSKE 5%) resulted in a CBR of 1:1.31 and was the least economical among the neem extracts followed by neem seed oil (NSO 0.5%) with 1:1.17; the most economical was neem leaf extract (NLE 20% with 1:1.15). Application of imidacloprid resulted in maximum cost-benefit ratio (1:1.34) followed by neem seed kernel extract 5% (1:1.31). A study in Ghana revealed that rotating sprays of tobacco extract (three applications) and cypermethrin (two applications) on cowpea generated greater economic return than five sprays of cypermethrin (Isman 2008).

2.6 Effect of plant part, solvents and conditions of applications on botanical extracts effectiveness

For botanical extracts to be regarded as effective against aphids, they should result in above 70% mortality which is considered as technically effective against aphids according to Gonzalez *et al.* (2011). The effectiveness of botanical extracts depends on the plant and plant part, solvent used and condition of application (Bahar *et al*. 2007; Tiwari *et al*. 2011). Botanical extracts from different plants tested on different aphid species under greenhouse, field and laboratory conditions showed variable aphid mortality ranging from 27% to 100% (Bahar *et al*. 2007; Iqbal *et al*. 2011; Sallam *et al*. 2009).

Stage of maturity of the botanical plant has an effect on the effectiveness of the botanical extracts. This has been related to the presence and absence of certain bioactive compounds in the mature and immature parts. The leaves of *Mentha piperita* and *Melea azedarach* gave higher cabbage aphid mortality than the seed extracts (Mekuaninte *et al*. 2011), indicating effectiveness of immature parts. Chiffele *et al*. (2009) showed that the immature leaves and immature fruits of *Melea azedarach* were effective against the fruit fly adults, *Drosophila melanogaster*, than the mature leaves and the ripe fruits. The leaves from the botanical plants may be sustainably harvested for use to prepare botanical extracts while the plants are allowed to grow and reproduce. The mature plant parts are known to have more health care related bioactive compounds like saponin which is cytotoxic and thus the plants become useful for

medicinal uses (Benny *et al*. 2015; Nicholson 2008). Therefore the same plant can be used for both medicinal and insecticidal purposes.

The extracts from the same plant may perform differently under field, net-house and laboratory conditions (Bahar *et al.* 2007; Gonzalez *et al*. 2011). There was higher mortality in the controlled environment (laboratory and net house) than in the field. This could be related to contact issues and application challenges where contact of insecticide, insect and surface may be limited on plants in the field than on bioassays. Aqueous extracts of chilli pepper, pyrethrum and castor oil leaves were effective against different aphid species, resulting in above 80% mortality under field conditions (Iqbal *et al*. 2011; Bahar *et al.* 2007). Application of aqueous extracts to control bean aphids in the field resulted in significant increase in yield of yard long beans (Bahar *et al*. 2007). Tobacco, garlic and neem aqueous extracts at 2% showed high efficacy against tea aphids (*Toxoptera auranti*) resulting in high aphid mortality of 66% – 96% under field conditions (Sohail *et al.* 2012). When aqueous extracts of healthy plants (leaves, roots and stems) of *Pongamia pinnata* extract, *Melia azedarach* and *Ipomoea fistulosa, Annona squanosa, Parthenium hysterophorus* and *Balanites aegyptiaca* were applied on artificially infested sugarcane woolly aphid on potted plants, aphid mortality was variable ranging from 67% - 92% (Wabale & Kharde 2010), indicating effectiveness of the various plant extracts on aphids mortality. The effectiveness of aqueous extracts also depends on dose and interval of spraying. According to Bhat & Yubak-Dhoj (2005), spraying *Melea azedarach* leaf extracts to control *B. brassicae* at a concentration of 1:5 w/v after 10 days and concentration of 1:10 w/v after five-day intervals resulted in significantly similar aphid population reductions in the field.

The solvent used to extract the botanical extracts also determines the effectiveness of the extracts, with aqueous extracts generally resulting in lower mortality (as low as 58%) than organic solvents extracts (Mekuaninte *et al* 2011; Ntonifor *et al.* 2010; Pavela 2009; Tiwari *et al.* 2011). Aqueous petroleum ether extracts from neem leaves, custard apple leaves and Mexican prickly pear sprayed against the cotton aphid (*Aphis gossypii)* significantly reduced aphid populations, resulting in above 80% aphid mortality (Chitra *et al.* 1997). When neem seed oil extract and pongam seed oil extract were sprayed on *M. persicae* on artificially infested tomato and cucumber greenhouse plants, 100% aphid mortality was obtained after 12 days (Pavela 2009). Ethanol extract (70%) of *Furcraea hexapetala* was effective against *M. persicae* giving a higher "in vitro" (73%) mortality than field (71%) on pepper and potato (González *et al.* 2011). There are fewer cases where aqueous extracts resulted in higher mortalities than alcohol

extracts for the same plant (Mekuaninte *et al.* 2011). This is related to the solubility of bioactive compounds where alcohol extracts may contain more bioactive compounds than water extracts which may have a synergistic effect on the target insects (Tiwari *et al.* 2011).

Commercial botanical insecticides cause various effects on insects both in the field and under laboratory assessments, ranging from acute toxicity, growth inhibition to feeding deterrence (Akhtar *et al.* 2008; Sallam *et al.* 2009). The commercial botanical insecticides (NeemAzal T/S, Trifolio S-forte and an extract of *Quassia amara* L.) were effective against the cereal aphids *Rhopalosiphum padi* (L.) and *Metopolophium dirhodum* under laboratory bioassays (Sallam *et al.* 2009). The bioactivity of some commercial botanical insecticides used as refined seed and bark extracts and crude extracts were reported to be highly toxic against the larvae of *Trichoplusia ni* and *Plutella unipuncta* showing strong growth inhibition, acute contact toxicity and significant feeding deterrence of the larvae (Akhtar *et al.* 2008). The frequency of application and concentration of botanical extracts is crucial in effectiveness of botanical insecticides (Bhat & Yubak-Dhoj 2005; Sallam *et al*. 2009). Botanical insecticides have short term effects on aphids and therefore frequent applications with lower concentrations may be a better option for farmers especially those into organic vegetable production. Due to their natural occurrence botanical plants may be readily available when needed.

It is important to note that the effectiveness of aqueous extracts and organic solvent extracts have variable effects on various species of aphids depending on solvent used, plant part, concentration and condition of application (whether applied on plant with residual effects or topical application on insects). The most effective plant part, concentration, solvent and application method should be used as per target insect pest.

2.7 Characteristics and effects of *Solanum panduriforme*

Solanum panduriforme Mey, ex Dunal is from the family Solanaceae. Other synonyms for *Solanum panduriforme* are *Solanum incanum* Linnaeus, *Solanum campylacanthum* Hochst. ex A. Rich *Solanum bojeri* Dunal, *Solanum delagoense* Dunal and *Solanum lichtensteinii* Willd (Lusweti *et al.* 2011; Matu 2008). The common names for *S. panduriforme* include yellow bitter apple, thorn apple, grey bitter apple, bitter tomato, Sodom's apple, Hottentot's poison bush, snake apple and poison apple (Hyde & Wursten 2011; Long 2005; Lusweti *et al.* 2011; Matu 2008).

Solanum panduriforme is a perennial herb or herbaceous soft wooded small shrub, often unarmed but occasionally with a few prickles (Hyde & Wursten 2011; Lusweti *et al.* 2011).The leaves are elliptic, entire or slightly wavy on the margins, dark green above, and paler and tomentose below. Flowers are pale to deep blue, mauve or purple. The fruit is mottled or striped green and white when unripe and yellow when ripe (Hyde & Wursten 2011; Matu 2008). The plant is found along roadsides, in grazing areas and in disturbed areas or abandoned fields, it is also found in various types of woodlands (Hyde & Wursten 2011; Lusweti *et al*. 2011; Masingi *et al.* 2008).

Solanum panduriforme has the properties of an ideal insecticidal plant. An ideal insecticidal plant should be perennial and widely distributed according to Silva-Aguayo (2009). If not widely distributed, it should be easily grown with reduced management. The plant parts used (leaves, flowers or fruits) should be removable without destroying the whole plant, and harvesting for botanical extractions should not mean destruction of the plants. Use of roots or the bark to extract the bioactive compounds should be avoided. Botanical plants should have additional uses for medicinal and veterinary uses with low economic values. *Solanum panduriforme* is also used for veterinary and medicinal purposes (Lusweti *et al*. 2011; Masingi *et al.* 2008). The active ingredients for ideal insecticidal plants should be effective at low rates (1:10 w/v) to protect non-target organisms and prevent excessive harvesting.

Solanum panduriforme contains glycolalkaloids, which are found in all parts of the plant with the highest concentrations in the fruit (Matu 2008). The main glycol-alkaloid solasonine has anti-feedance effects on insects. Alkaloids extracted from chilli are effective against the cabbage aphid *B. brassicae* (Habimana & Hakizayezu 2014) under laboratory conditions and alkaloids from a botanical plant sabadilla lily (*Schoenocaulon officinale*), a tropical lily that grows in Central and South America are used as active ingredients in commercial botanical insecticides (Dubey *et al.* 2010). There is a high possibility that alkaloids from *S. panduriforme* may be used as an active ingredient to develop a commercial botanical insecticide. The ripe berries reportedly used by farmers can be a good source for extracting the alkaloids since highest concentrations are reportedly found in the fruits (Benny *et al.* 2015; Matu 2008).

Most secondary metabolites from Solanaceae have insecticidal properties that affect insects from most orders (Chowański *et al.* 2016). *Solanum* species extracts have been shown to be toxic to a number of insects, which include leaf-eaters and sap suckers such as aphids and

thrips, stored products insects, mosquitoes, termites, flies, cockroaches and some predatory species. The secondary metabolites have a wide spectrum of activity on insects at the cellular, tissue and organism level (Table 2.1). Their actions disturb the cellular and physiological processes responsible for maintaining homeostasis. They can cause sub-lethal changes within various tissues and organs leading to death of insects. The secondary metabolites can also lead to reduced fecundity, reduced viability and deformities, thus disturbing insect development and they may act as repellents. The bioactive compounds may act synergistically either from the same plant or from different plants; peel extracts of *Helix aspersa* inhibited feeding more effectively than solutions of pure glycoalkaloids (Chowański *et al.* 2016; Yi *et al*. 2012).

Table 2. 1: Acute and sub-acute effects of Solanaceae extracts on aphids

Extract	**Activity**	**Aphid Species**
Capsicum frutescens L. leaf extract	Antifeedance, deterrence, reduced infestation	*Brevicoryne brassicae* (L.)
Lycium cestroides Schltdl. leaf extract	Antifeedance, settling inhibition, contact toxicity	*Myzus persicae*
Nicotiana tabacum L. leaf extract	Antifeedance, deterrence	*Brevicoryne brassicae*

Adapted from Chowanski *et al.* (2016)

According to Chowański *et al.* (2016), the effects of Solanaceae secondary metabolites on insects can be on growth, metabolism, behaviour and reproduction as indicated in Fig. 2. 1

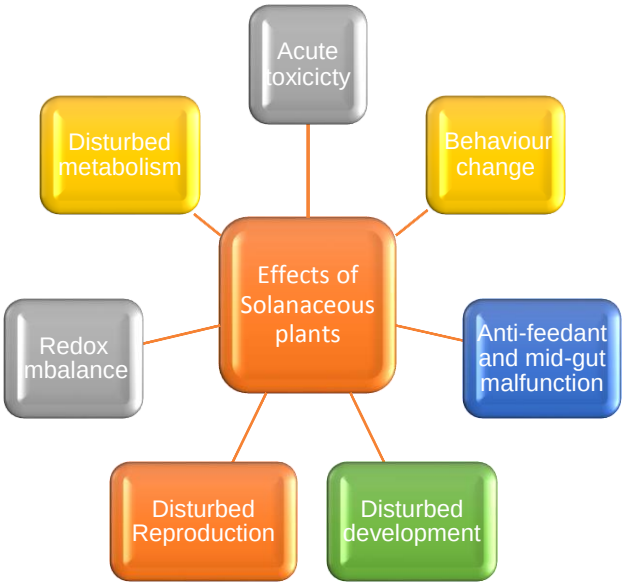

Figure 2.1 Effects of Solanaceae metabolites (Adapted from Chowanski *et al.* (2016)

Alkaloids are the most common biologically active compounds within the Solanaceae family. Extracts from Solanaceae plants cause death of target insects due to their acute toxicity even in very low doses (Chowanski *et al.* 2016). The compounds mainly disturb the structure of biological membranes and cellular metabolism of insects acting as cellular membrane-disrupting factors or inhibitors of acetylcholinesterase activity. Nicotine from tobacco (*Nicotiana* spp.), a Solaneceae plant, exhibits high insecticidal activity, mostly because it mimics acetylcholine and intensifies synaptic transmission (Chowanski *et al.* 2016). The bioactive compounds from Solaneceae plant extracts also promote antioxidant mechanisms such as the activation of the antioxidant enzymes causing oxidative stress that affect processes such as the peroxidation of membrane lipids, the disruption of mitochondrial membrane potential or protein damage. Some alkaloids from Solanaceae plants disturb cellular structure as indicated by numerous data concerning the cytotoxic activity of the plant extracts mostly related to cancer in human beings (Chowanski *et al.* 2016). Alkaloids are carcinogenic and therefore it is important for users to wear protective clothing when processing and applying botanical extracts with alkaloids. Alkaloids may interact with the endocrine system of pests, especially altering juvenile hormone activity thus disturbing insect development (Bogran *et al*. 2011). Alkaloids from sabadilla affect nerve cell membrane action, causing loss of nerve cell membrane action, resulting in loss of

nerve function, paralysis and death; some insects may immediately be killed, while others may survive in a state of paralysis for several days before dying (Bogran *et al*. 2011). Glycoalkaloids, through their similarity to sterols, may affect insect moulting and development processes that are regulated by steroidal hormones such as ecdysone and they can increase concentration of certain ions in the cell resulting in redox imbalance (Chowanski *et al.* 2016; Gonzalez 1997). Phenolic compounds and flavonoids may also exhibit anti-feedance and repellent effects on insects; they were reported to modulate the feeding behaviour of the pea aphid (Golawska *et al.* 2008a; Golawska *et al.* 2008b).

Many leaf and fruit extracts from Solanaceae plants have been found to be effective against aphids and other insects through disturbing various activities and behaviours of the insects as shown in Table 2.2. For example the extracts of leaves and fruits of *Solanum aculeatissimum* have a repellent effect on *B. brassicae* (Nicholson 2008). Solanaceae plants which include *S. panduriforme* have been reported to have great potential for developing new crop protection chemicals, more compounds or mixtures of their compounds have been identified as pesticides control agents (Chowański *et al.* 2016).

Table 2.2: Acute and sub-acute effects of Solanaceae pure compounds on aphid species

Compound	**Effect**	**Aphid Species**
2-undecanone, 2-dodecanone, 2-tridecanone, 2-pentadecanone	Increased adult mortality	*Aphis craccivora*
laxumin A & B, Solanine, chaconine, luciamin	Decreased adult survival, antifeedance,	*Schizaphis graminum*
Nicotine	Mimicked acetylcholine and interacted with nicotinic acetylcholine receptors	Most aphids species (*B. brassicae, M. persicae)*
α-solanine, α-chaconine	Reduced fecundity and feeding of adults, reduced weight, increased mortality of nymphs	*Myzus persicae*
Capsaicin	Increased efficiency of synthetic pesticide (Neemix, Pyronyl, M-pede)	*Myzus persicae*

Adapted from Chowanski *et al*. (2016)

2.8 Effect of extraction techniques and methods on bioactivity of botanicals

Extraction is the separation of active components of plant tissues using selective solvents through standard procedures (Tiwari *et al*. 2011; Sasidharan *et al*. 2011). Since extraction is the first crucial step in the analysis of botanical plants it is important to extract the desired chemical components from the plant materials for further separation and characterisation (Sasidharan *et al*. 2011; Sajfrtova *et al.* 2013). Plant based botanical extracts may be derived from the bark, leaves, flowers, roots, fruits or seeds containing the bioactive compounds (Sasidharan *et al*. 2011; Tiwari *et al.* 2011). Fresh or dried plant materials can be used as a source for extraction of insecticidal active secondary plant components but dried plant materials are preferred because fresh plant materials are fragile and perishable resulting in faster deterioration than dried materials (Azwanida 2015). Fresh and dried leaves of *Moringa oleifera* showed no significant effect on quantity and quality of phenolics though dried samples contained higher flavonoids. However it has been observed that dried plant materials need to be treated in a way that ensures compounds of interest are efficiently liberated into solution (Sasidharan *et al*. 2011). Certain extracts from dried plant materials may become ineffective due to incomplete

extraction and thus require more complicated extraction methods like high performance liquid chromatography (HPLC) to be performed using guard columns and analysis to examine if the extracts contain the compounds of interest.

The basic operations for extraction include steps such as pre-washing, drying of plant materials or freeze drying and grinding to obtain a homogenous sample. The basic principle in extraction is to grind the plant material (dry or wet) to a finer product (Sasidharan *et al*. 2011; Tiwari *et al* 2011). This increases the surface area for extraction and contact of sample surface with the solvent system thus improving the kinetics of analytic extraction, thereby increasing the rate of extraction and the amount of active components (Azwanida 2015; Tiwari *et al*. 2011). Many different preparations with different components can be obtained from a specific botanical plant depending on the plant part used, the solvent used for extraction and the extraction procedure used to separate the active portions of plant tissues (Chiffelle *et al.*2009; EFSA 2009; Sasidaharani *et al.* 2011; Tiwari *et al* 2011). The origin of the plant, degree of processing and particle size may determine the quality of the extract from the same plant.

To extract the bioactive compounds, the desired plant parts are soaked in the selected solvent; the choice of solvent is influenced by the targeted compounds or the specific nature of the bioactive compound being targeted (Sasidharan *et al*. 2011; Tiwari *et al.* 2011). The solvent used for extraction should neither interfere with the targeted compounds nor the effectiveness of extracts when used in assays (bioassays, plant assays, phytochemical screening). A good solvent should result in low toxicity to avoid potential health hazards of the extract and it should be easily evaporated (Sasidharan *et al.* 2011). The main solvents that are used for extraction are water, methanol, ethanol, acetone, ether, chloroform or a mixture of organic compounds and water which may be referred to as aqueous alcohol extracts or aqueous ethanolic extracts if ethanol is used (Azwanida 2015; Tiwari *et al.* 2011; Sasidharan *et al*. 2011). Water, the universal solvent, has been traditionally used to produce aqueous extracts of the desired plant material for medicinal, veterinary and plant protection use (Tiwari *et al.* 2011). Water extracts contain significant soluble phenols which are important as antioxidant compounds. When alcohol is used, it penetrates the cellular membranes thus extracting the intracellular components. Ethanol is reported to be the best solvent for flavonoids and when water is added to the ethanol up to 70%, the polarity of ethanol increases resulting in better quality extracts than when used alone (Azwanida 2015; Tiwari *et al.* 2011). Ethanol extracts are known to be

more active than aqueous extracts due to presence of polyphenols in cases where polyphenols are responsible for biological activities (Sasidharan *et al*. 2011; Tiwari *et al.* 2011). Polyphenols are degraded in water but remain inactive in methanol or ethanol. Methanol is more polar than ethanol but due to its cytotoxic nature, it is unsuitable for extraction of certain compounds that may have human health hazards (Tiwari *et al.* 2011).

Different extraction methods and procedures have been used to extract medicinal and insecticidal bioactive compounds from plants. The extraction methods include maceration, homogenization/percolation, decoction, Soxhlet or hot continuous extraction, solvent assisted/sequential /serial exhaustive, and microwave assisted extraction; maceration, homogenization and decoction are the easiest, simplest and most used methods (Azwanida 2015, Handa *et al.* 2008; Sasidharan *et al.* 2011). Decoction is only suitable for extracting heat-stable compounds and hard plants materials like roots and barks (Azwanida 2015; Handa *et al*. 2008; Tiwari *et al.* 2011). The Soxhlet extraction method requires a smaller quantity of solvent compared to maceration (Azwanida 2015; Handa *et al*. 2008; Sasidharan *et al.* 2011). The disadvantages of the Soxhlet extraction are associated with exposure to hazardous and flammable liquid organic solvents, which have potential toxic emissions during extraction. The solvents used in the extraction system should also be highly pure and these are expensive (Azwanida 2015; Yang *et al*. 2004). The solvent assisted extraction method is used to obtain polar and non-polar extracts from the same sample by extracting plant parts successively with solvents of variable polarities to generate extracts with variable solubility characteristics.

The quantity and quality of bioactive compounds is more influenced by the solvent used to extract than the extraction method (Yang *et al.* 2004). The solvents used in processing the botanical extracts are more critical on the extract quality and quantity than the method used for extraction. The extraction techniques do not necessarily determine the chemical quantity and therefore the quality and quantity of extracts from sequential/solvent assisted extraction, Soxhlet extraction, gas chromatography (GC) or high performance liquid chromatography (HPLC) are quite similar (Yang *et al*. 2004). The extracts from gas chromatography were comparable to the quantity of extracts obtained by hexane using Soxhlet extraction (Sajfrtová *et al*. 2013).
Other factors which influence composition of secondary metabolites from the different methods are period of extraction, temperature for extraction, solvent concentration, solvent to sample ratio and the nature of the plant material (fresh versus dried and if dried whether air, oven or microwave dried, grounded or powdered). The choice of solvent to use play a critical role in the

effectiveness of the extracts though temperature variations improve the extraction process and reduce the volume needed for extraction (Azwanida 2015; Tiwari *et al*. 2011).

Boiling or decoction of *Centella asiatica* at 90 °C resulted in increased phenolic content and antioxidant activities for medical use (Azwanida 2015). Medicinal studies on extraction of *Psidium guajava* leaves using maceration and homogenization with ethanol and aqueous-alcohol extracts (4:1 v/v) resulted in highest extraction yield and a maximum presence of phytoconstituents (alkaloids, saponins, carbohydrates, tannins and flavonoids) compared to non-polar solvents like ether and chloroform (Azwanida 2015); polar solvents were reportedly more effective in the extraction of bioactive molecules from *Psidium guajava*. Different solvents used at 1:10 w/v sample to solvent resulted in more total phenolics from aqueous-alcoholic extracts (70 % acetone and 70 % ethanol). Maceration used to extract *Moringa oleifera* powder with 70% ethanol at 1:40 w/v resulted in highest phenolics and flavonoids content compared to Soxhlet extraction and homogenization using similar solvent (Azwanida 2015). Soxhlet extraction of *Azadirachta indica* leaf powder in methanol at 1:5 w/v resulted in numerous phytochemicals (Azwanida 2015).

The few farmers who use botanical extracts to control insects generally use aqueous extracts from homogenisation and maceration methods, though some use the decoction method. The resulting solutions are filtered with fine mutton cloth and used directly with or without further dilution depending on initial concentration used. When organic solvents are used the extract solution is also filtered and usually dried under reduced pressure to remove the organic solvent and to obtain a crude extract or dried concentrated absolute extract (Dadang *et al.* 2009; Mekuaninte *et al* 2011; Srivastava & Guleria 2003; Tiwari *et al* 2011). The Sohxlet method is mainly used for extraction when using organic solvents. If the dried crude extracts are not used immediately they are kept under low temperature in the refrigerator until used. The absolute or dried extract may be re - dissolved in an appropriate solvent like acetone (Tiwari *et al.* 2011) or it may be further diluted with water and used as a spray.

Acetone is used to dissolve the dried crude extracts without affecting the effectiveness of the dried crude extract on the insects (Ntonifor *et al*. 2009; Tiwari *et al.* 2003). Acetone has been reportedly used as an anaesthetic to harvest and handle large aphid (*M. persicae*) populations in assessments of persistent virus (potato leaf roll) and non-persistent virus (papaya ring spot) transmission assays (de Souza *et al*.1993). Use of acetone was 8 – 10 times faster and 30 %

more efficient than the mechanical handling using camel hair brush to harvest the aphids. Acetone enabled collection and handling of the smallest nymphs with no significant differences in transmission from acetone harvested and mechanical harvested aphids. This shows that acetone does not interfere with the effectiveness of botanical extract on the aphids

Researchers have tested the effectiveness of botanical extracts from aqueous and organic solvents on a number of insects, including aphids. Aqueous extracts from orange peels, garlic leaves or bulbs, bitter gourd, marigold, hot pepper, tobacco, neem (leaves, kernels or seed), poison apple berries soaked in water for 24 h – 72 h (Iqbal *et al.* 2011; Mhazo *et al.* 2011; Ntonifor *et al.* 2010; Stevenson 2010) have resulted in variable effectiveness on target insects under laboratory and field conditions. Aqueous extracts from spices (*Piper guineense, Afromomum melengeta and Afrostrax kamerunensis*) extracted using homogenisation method showed anti-feedance, lethal and reduced developmental effects on third instar larvae of *Plutella xylostella* (Ntonifor *et al.* 2010). Hexane and ethanol extracts from *Mutingia calabura* prepared using maceration were also effective against the larvae and pupae of diamond back moth (Bandeira *et al.* 2013). Fresh plant parts (leaves, roots and stems) from *Pongamia pinnata, Impomea fistulosa, Annona squamosa* were aqueous extracted using maceration resulting in effective control of sugar cane woolly aphids under greenhouse conditions (Wabale & Kardhe 2010). Sohail *et al*. (2012) used the decoction method to prepare neem seed and neem powder extract which was used to effectively control tea aphids (*Toxoptera auranti*) in tea nursery plants an indication that the seeds gave effective heat liable compounds.

The selection of an extraction technique or method depends on operational factors which include, but not limited to instrument cost and availability, labour cost, operational cost and consumable cost (Ong 2004; Yang *et al.* 2004). Because of these factors maceration, homogenisation and sequential or solvent assisted methods were the preferred methods in this current study to extract aqueous extracts, ethanol extracts and dried crude extracts.

2. 9 Importance of separation, identification and characterization of phytochemicals

Plant extracts occur as a combination of various bioactive compounds or phytochemicals with different polarities (Sasidharan *et al*. 2011). The initial crude extracts using most of the extraction methods mentioned in Section 2.8 contain complex mixtures of many plant metabolites, such as alkaloids, glycosides, phenolics, terpenoids and flavonoids (Azwanida 2015). Some of the initial extracts may be ready for use as insecticides and medicines but some

may need further processing to separate into desirable bioactive compounds. The natural plant materials contain significant levels of strongly binding components which result in variations in phytochemical constituents from different samples. It is suggested that more than one method be used to separate and identify the bioactive compounds using chromatography and non-chromatography techniques which may be combined (Sasidharan *et al*. 2011).

Non-chromatographic techniques facilitate identification of bioactive compounds and these include immunoassays, phytochemical screening assays, and Fourier-transform infrared spectroscopy (FTIR) (Prashanth & Krishnaiah 2014; Sasidharan *et al*. 2011; Singh & Chauhan 2014). When phytochemical screening assays are used, the presence of phytochemicals or bioactive compounds is confirmed based on tests of colouration and precipitation (Sasidharan *et al*. 2011). Phytochemical screening assays are simple, quick, and inexpensive procedures that give quick answers to the various types of phytochemicals in a mixture; they are an important tool in bioactive compound analysis (Sasidharan *et al*. 2011). This method has been used to identify bioactive compounds in neem leaves and berries (Prashanth & Krishnaiah 2014; Singh & Chauhan 2014). Phytochemical screening assays are ideal to identify the bioactive components from *Solanum panduriforme* leaves and berries aqueous and alcohol extracts.

Chromatography techniques are used to obtain and identify pure compounds and these include thin layer chromatography (TLC), column chromatography, flash chromatography and high performance liquid chromatography (HPLC) (Sasidharan *et al*. 2011). Identification of compounds is based on the comparison of mass spectra and retention indices with published or known results and where possible with authentic genuine compounds (Sajfrtová *et al.* 2013; Sasidharan *et al*. 2011). The pure compounds are then used for the determination of structure and biological activity. Natural products may be isolated after evaluation of an extract in a biological or plant assay in order to fully characterize the active entity. The biologically active entity is often present only as minor component in the extract; chromatographic techniques like HPLC may be used to rapidly process multicomponent samples on both an analytical and preparative scale (Sasidharan *et al*. 2011). Challenges associated with chromatographic techniques like HPLC are that instruments comprise a solvent delivery pump, a sample introduction device such as an auto-sampler or manual injection valve, an analytical column, a guard column, detector and a recorder or a printer, which are not always available. For correct identification, the analytical chemist should also be highly knowledgeable to choose the appropriate conditions, such as the proper mobile phase, flow rate, suitable detectors and

columns to get an optimum separation otherwise identified compounds may lead to a wrong conclusion. It is therefore important for the analytical chemist to work closely with the entomologist carrying out the chemical analysis repetitively as much as possible and at the same time the entomologist testing the compounds against target insects.

Separation and determination of the most bioactive compound is a challenge because the compounds have to be purified by using several chromatographic techniques and various purification methods to isolate bioactive compound(s). As mentioned earlier, extracted compounds from the same extraction solvent are fairly close in terms of quality and quantity regardless of extraction and separation method used.

2.10 The future of botanical insecticides for aphid control

The use of plant extracts to control destructive insect pests or disease vectors is not new. Many botanical insecticides have been known and used for hundreds of years but were replaced by synthetic insecticides in the 1950s (Buss & Park-Brown 2009; Silva-Aguayo 2009). Rotenone (*Derris* spp.), nicotine and pyrethrins have been used for a considerable time in small-scale subsistence agriculture. It is reported that some farmers in Zambia and Malawi have been traditionally using over 20 different pesticidal plant species which include *Tephrosia vogelii*, neem (*Azadirachta indica*), *Mucuna pruriens*, *Bobgunnia (Swartzia) madagascarensis*, *Euphorbia tirucali*, *Vernonia amygdalina*, *Tithonia diversifolia*, and *Solanum panduriforme* (Nyirenda *et al.* 2011; Stevenson 2010) but with challenges of effectiveness and adoption as mentioned in section 2.5.

According to Prakash *et al*. (2008), botanical pesticides possess an array of properties which make them important alternatives to minimise or replace the use of synthetic pesticides. The advantages of botanical insecticides according to Silva-Aguayo (2009) and Buss & Park-Brown (2009), are that they break down fast since they degrade rapidly in sunlight, air and moisture, they break down readily in soil and are not stored in plant or animal tissue, which means crops may be harvested and used shortly after spraying. One of the most desirable properties of neem is its low degree of toxicity. It is considered almost non-toxic to humans and animals, and is completely biodegradable according to Lajeunesse (2001), making it a very good option for integrated pest management.

Botanical pesticides, however, do have some disadvantages (Silva-Aguayo 2009). Most of the products are not truly insecticidal. Most plant species used for plant protection exhibit an insect deterrent rather than insecticidal effect; hence their effect is slow and it is the reason why some farmers believe they are ineffective. They are rapidly degraded by ultra violet light resulting in short residual action. There is need to make frequent well timed botanical spray applications to achieve effective control of target insect pests. Not all plant insecticides are less toxic than synthetic insecticides; some botanicals like nicotine and rotenone are relatively toxic, just like some organophosphates and carbamates; inhalation and skin exposure to nicotine preparations can cause death at LD 50 of 50 mg kg^{-1} (Bogran *et al*. 2011; Isman 2006). Rotenone is similar in toxicity to the common synthetic insecticides carbaryl and diazinon (Buss & Park-Brown 2009) and may be hazardous to the environment, non-targets and humans, if not properly handled. Most of the botanical insecticides have no established residue tolerances and not much work has been carried out on efficacy which involve toxicological studies. They are used without any legal registration because they are not commercially produced (Isman 2006; Isman 2008). In most African countries data on regulated botanical insecticides are not readily available, unlike in European countries, and this may affect users of botanical insecticides and consumer safety as well as affecting the environment.

Botanical insecticides have been found to have economic benefits over synthetic insecticides, especially in developing countries, where farmers cannot afford synthetic insecticides (Dobson *et al*. 2002; Isman 2006; Sinzogan *et al.* 2006; Stevenson *et al*. 2012). Botanical insecticides can be prepared from locally available plants and used in different ways by different users as dusts, ready to use solutions, emulsifiable concentrates, flowable concentrates or soluble concentrates. When used as ready to use solutions, they are readily, easily and cheaply available than synthetic insecticides (Chowański *et al.* 2016; Isman 2006; Isman 2008). Farmers in Cameroon have used plant extracts of local plant species either alone or mixed with conventional imported synthetic insecticides for cocoa production to reduce production costs (Coulibaly *et al.* 2002; Padi *et al.* 2000). Where synthetic insecticides are affordable to growers through government subsidies, limited literacy and a lack of protective equipment may result in thousands of accidental poisonings (Isman 2006; Isman 2008).

According to Prakash *et al*. (2008), the future of botanical pesticides in agriculture depends on (1) search for plants with insecticidal properties; (2) isolation, identification and evaluation of the active components; and (3) synthesis of the active components for commercial use. Commercial

botanical insecticides may be of great advantage to organic farmers who have to meet demands of organic food consumers who are prepared to pay a higher price for organic produce; however resource poor farmers may not afford commercially produced organic insecticides. Isman (2006) suggests that the research community should focus its attention on the development and application of known botanicals rather than screen more plants. Stevenson *et al*. (2012) suggest that pesticidal plants should be used with limited processing that can be carried out by the farmers to enable farmers to afford and adopt the use of locally and easily available plants. Other researchers suggest that studies on the formulation of botanical insecticides, their efficacy and effects on the environment are equally important for adoption and use of the botanicals (Ntalli & Menkissoglu-Spiroudi 2011; Solliman *et al.* 2005).

The future of botanical insecticides has also been highlighted by other researchers who suggested the need to scale up studies to understand the mechanisms of extraction of botanical insecticides even from some common vegetables and fruits like garlic, tomatoes, grapes, gourd and citrus which also contain secondary bioactive compounds especially if the bioactive parts are the waste products that may be used at the household level to replace harmful insecticides (Bahar *et al*. 2007; Iqbal *et al.* 2011; Olabniri *et al.* 2014). Others recommended that local plants with insecticidal properties should be made available to farmers after development of scientific formulations (Wabale & Kharde 2010). Quick bio-prospecting of the available traditionally used flora to document pesticidal plants in order to check future cases of bio-piracy and to establish sovereign right on the botanical pesticides developed from such plants was recommended by Dubey *et al.* (2010). Toxicity research on plant extracts has been reported to be crucial in the search for new bioactive compounds that may be synthesized and used commercially for future use (Chowański *et al.* 2016; Sajfrtova *et al*. 2013).

To produce effective botanical insecticide formulations, it is important to follow the steps and phases for discovery of botanical insecticides which include extraction and evaluation of the plant extracts using bioassays and plant assays. A summary for screening of the plants extracts to discover the bioactive compounds are illustrated in Fig 2.2. Mclaughlin *et al.* (1998) and Stell *et al.* (2013) reported that for botanical plants to be accepted and incorporated into legitimate long term practices, preliminary work on the usefulness of the plants as insecticides and repetitive assays to evaluate the bioactive compounds from the plant parts is integral to the discovery of the bioactive compounds.

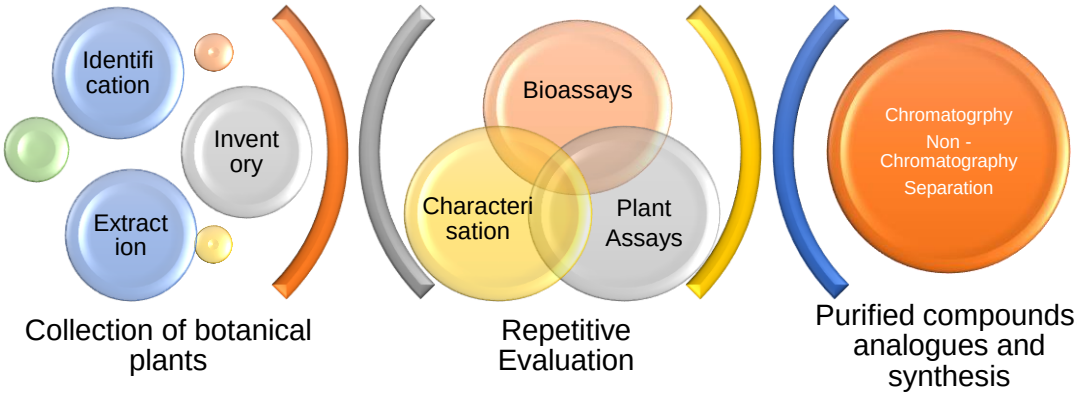

Fig. 2.2 Phases for screening of plant extracts for bioactive compounds discovery and use Adapted from Chen (2009); Sasidharan *et al*. (2011)

The pesticidal plants are basically identified by the farmers through their indigenous knowledge practices and they are mostly collected from the wild (Stevenson *et al*. 2010). The repetitive assays to evaluate effectiveness of the pesticidal plants are carried out by researchers using bioassays under laboratory conditions and plant assays under screen-house and field conditions (McLaughlin *et al.* 1998). The field assays may also involve farmers as on-farm research trials. The researchers use the best method to separate the bioactive compounds, depending on operational factors like instrument cost and availability, labour cost, consumable costs and other operational costs (Tiwari *et al*. 2003; Yang *et al*. 2004). The industry synthesises the bioactive compounds into an affordable commercial botanical extract for use by farmers (Chowanski *et al*. 2012; Isman 2006). The yield of cabbages treated with botanical crude extracts from *Azadirachta indicasee, Dodonae angustifolio* and *Cymbopogon citrates* was not significantly different from that of Diazinon 60 E.C; there was no significant differences in the cabbage aphid populations (Shiberu & Negeri 2016). The reseachers recommended that these botanicals should be considered for cabbage aphid management in Ethiopia.

Recent studies in Africa suggest that indigenous knowledge and traditional practice can make valuable contributions to domestic food production in countries where strict enforcement of pesticide regulations is not practical (Isman 2008). The use of botanicals will be of great benefit in developing countries for many years to come especially, on crops that are grown for home consumption (Isman 2006; Dubey 2010). However it is important to come up with relatively cheaper alternatives that can be afforded by resource poor farmers. For example dried and ground leaf powder formulations from any effective part as long as full instructions on use are

available may be commercially produced to be easily available, accessible and used directly without much processing by the farmers.

According to El-Wakeil (2013), the use of botanical insecticides by farmers may be affected by raw material availability, standardization of botanical extracts with complex mixture of bioactive compounds, solvent types, plant species and part of plant, state registration and market opportunities for botanical pesticide. Discovery and long term use of botanical plants therefore requires cooperation and collaboration of farmers, researchers industry and the state. This research is carried out to gather information on *Solanum panduriforme* effectiveness, an insecticidal plant that was identified by farmers. The information is valuable to inform farmers on how they can adopt and use the plant in organic insect pest management and integrated pest management approaches in their fields so they can achieve food security and also to enable industry to develop an affordable commercial *S. panduriforme* based botanical insecticide.

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CHAPTER 3: GENERAL MATERIALS AND METHODS

The research was carried out at the University of Venda (22.9761° S, 30.4465° E) and at the University of Swaziland (26.4791° S, 31.3068° E). All the plant materials used in the research were collected from grazing areas and abandoned fields around the University of Venda, Limpopo Province, South Africa.

3.1 Extraction methods and procedures

3.1.1 Plant materials

The plant parts used were young leaves from young plants, ripe berries and unripe berries from *Solanum panduriforme* mother plants (Fig. 3.1). The ripe berries were used as both fresh and powdered, young leaves were used as powdered and unripe berries used as fresh. The young leaves were harvested from vegetative young plants by snipping off 2 – 3 young leaves and shoots of the plant before drying them in the greenhouse. Ripe berries were sliced into half before drying; they were not chopped to prevent losing some of the juices before and during the drying process. Fresh ripe berries and fresh unripe berries were sliced in half before extraction. The dried plant parts (ripe berries and young leaves) were ground into fine powder using an electric grinder (Retsch zm 200) (Fig. 3.2).

Figure 3.1: *Solanum panduriforme* mother plants (a, b), dried young leaves (c), dried ripe berries (d), fresh ripe berries (e), and fresh unripe berries (f)

Figure 3.2: Electric grinder used for grinding dried leaves and dried berries

3.1.2 Extraction procedures

The extraction procedures used to produce aqueous and ethanol extracts were plant tissue homogenization for the powders (Tiwari *et al.* 2011) and maceration for the fresh berries (Sasidharan *et al.* 2011). The solvent-assisted extraction technique (Soliman *et al.* 2005) using hexane and diethyl ether was used to produce dried crude extracts. The solvents used were water for aqueous extracts, 60% ethanol for alcohol extracts and hexane and diethyl ether for the dried crude extracts.

3.1.3 Aqueous extraction from leaf powder, berry powder, fresh ripe berries and fresh unripe berries of *Solanum panduriforme*

Powders (Fig. 3.3) from dried leaves and dried berries were separately mixed with distilled water at 10 g of plant material per 400 ml water to prepare dried leaf and dried ripe fruit aqueous extracts using homogenization extraction (Tiwari *et al.* 2011). Each mixture was shaken vigorously for 5 min and left to stand for 24 h after which it was shaken again. The mixture was filtered through a Whatman filter paper (number 9) to produce the aqueous extracts from powder preparations. The maceration procedure was used to process berries. Fresh half sliced ripe berries and unripe berries (125 g) were kept in 500 ml water in tightly sealed containers for 48 h with frequent agitation to dissolve soluble matter, by slightly shaking the containers (Fig. 3.3). The mixture was filtered through a fine mutton cloth.

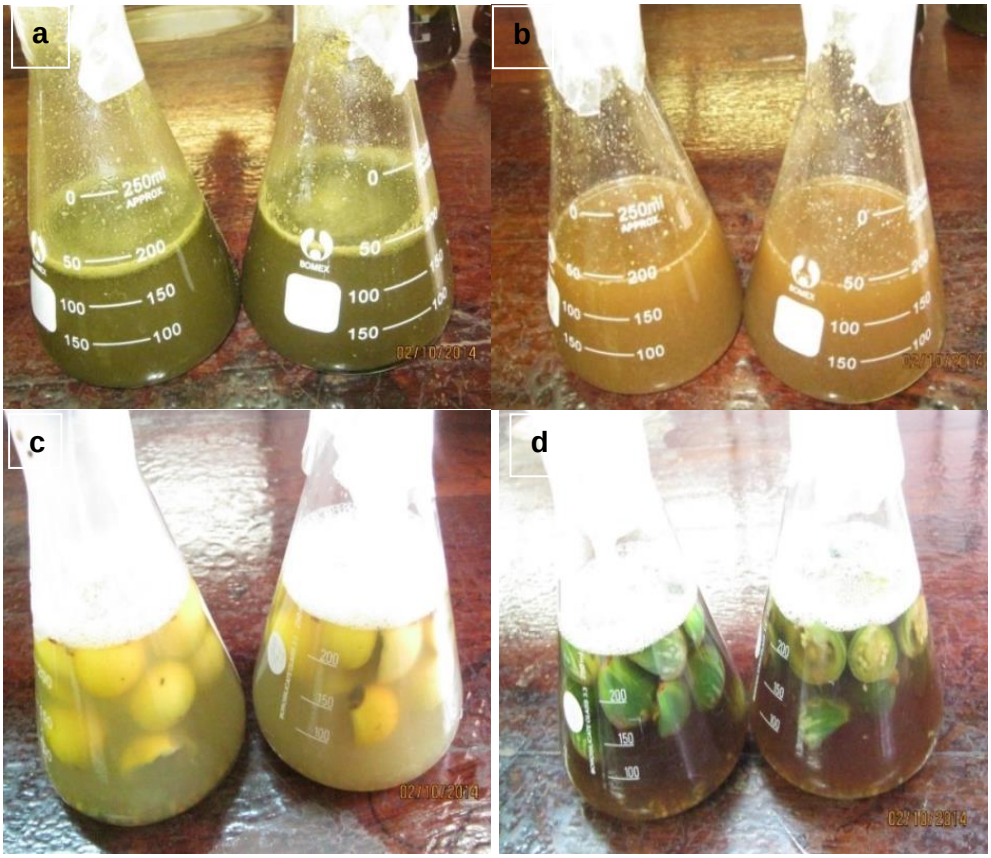

Figure 3.3: Maceration and homogenisation extraction of *S. panduriforme* extracts
Leaf powder (a) and berry powder (b) using homogenisation technique
Fresh ripe berries (c) and fresh unripe berries (d) using maceration technique

3.1.4 Alcohol extraction from leaf powder, berry powder, fresh ripe berries and fresh unripe berries of *S. panduriforme*

Powders from dried leaves and dried berries were soaked at 10 g powder in 500 ml 60% ethanol (Tiwari *et al*. 2011). The mixture was shaken vigorously for 5 min and left to stand for 24 h after which it was filtered through a Whatman filter paper (number 9). Fresh half sliced ripe berries and unripe berries (125 g) were soaked in 500 ml 60 % ethanol (Bahar *et al.* 2007; Wabale & Kharde 2010). The mixtures were kept at room temperature for 48 h in a tightly sealed container with frequent agitation by slightly shaking the containers to dissolve the soluble matter. After 48 h the solutions were filtered through fine mutton cloth.

3.1.5. Hexane and diethyl ether extraction of leaf powder, berry powder, fresh ripe berries and fresh unripe berries of *S. panduriforme* using sequential or solvent assisted extraction technique

The sequential extraction or solvent assisted extraction technique (Fig. 3.4) was used to obtain dried crude extracts from a single sample using solvents with variable increasing polarities (water, hexane and diethyl ether). The residue of the previous extraction was used as the feed for the next extraction (Jeyaseelan *et al*. 2012). Leaf powders and berry powders were each separately mixed with distilled water (10 g powder per 500 ml) to prepare dried leaf and dried fruit aqueous extracts using the homogenization extraction. Fresh ripe berries and fresh unripe berries were mixed separately with distilled water (100 g fresh sliced berries in 400 ml water) to obtain fresh fruit aqueous extracts using the maceration procedure. The aqueous extracts were water pressure filtered using Whatman paper number 1 after 24 h for powder extracts using the homogenisation procedure and after 48 h for the fresh berries extracts using the maceration procedure. The aqueous extracts were then mixed with 400 ml hexane and shaken to produce two columns: water and hexane. The hexane extract was collected from the bottom of the glass funnel leaving the water extract on top of the flask. The remaining water extract was again mixed with 400 ml diethyl ether to yield water and diethyl ether extract columns. The diethyl ether extract separated from the water extract was again collected from the bottom of the funnel. The remaining water extracts were kept in the refrigerator until use. The hexane and diethyl ether extracts were dried in a flow cabinet to obtain dried crude extracts or absolute extracts which were transferred into small vials and weighed to get the extract amount from each extraction. The vials with the extracts were kept in the refrigerator for use in topical bioassays.

Figure 3.4: Solvent assisted extraction (a) pressurised water filtering, (b) Separating hexane and diethyl ether extracts (c) Hexane and water extracts (d) Ripe berries hexane and di-ether extracts

3.2 Insect rearing and production of plants for bioassays and plant assays

The cabbage aphids (*Brevicoryne brassicae*) used in the preliminary bioassays were collected from naturally infested brassica crops that were grown in the field at the University of Venda. The aphids for subsequent bioassays were reared on rape plants in the field plants and in the screen-house in pots at the University of Swaziland; they were made up of both adults and nymphs. Rape plants for producing leaf cuttings for bioassays and for plant assays were also raised in pots in the screen house and in the field.

3.3 Laboratory bioassays

Desk top bioassays for natural plant products were used (McLaughlin *et al*.1998) to assess the effectiveness of botanical extracts from the young leaves, unripe berries and ripe berries of *Solanum panduriforme* on the mortality of the cabbage aphid *B. brassicae*. The leaf dip

bioassays (Moslemi *et al.* 2011; Sallam *et al.* 2009), aphid and leaf dip bioassays (Atteyat *et al.* 2012), aphid dip bioassays (Chandrasena *et al.* 2011; Kranthi 2005) and topical application bioassays (Coelho *et al*. 2006; Kranthi 2005) were used. Choice and no choice bioassays were used to determine the effect of the extracts on aphids' behaviour.

3.3.1 Leaf dip or leaf cuttings residual bioassays

Leaf squares (4 cm x 4 cm) were separately dipped in the *S. panduriforme* extracts and controls (water and malathion) for 10 sec (Moslemi *et al.* 2011; Sallam *et al.* 2009). The cuttings were air dried for 5 min before each leaf cutting was placed separately upside down in a Petri dish lined with moist kitchen paper to reduce desiccation. Ten to 15 aphids were released on each leaf cutting by means of a camel-hair brush. The number of dead aphids from each Petri dish was recorded 12, 24 and 36 h after releasing the aphids. Aphids which did not move after a gentle touch were considered dead.

3.3.2 Aphid and leaf dip bioassays

Detached leaf cuttings were used (Atteyat *et al*. 2012) to evaluate the effect of the botanical extracts. The leaf cuttings were checked under a microscope to ensure that approximately 20 similar wingless adult aphids remained on each leaf cutting. The leaf cuttings with the aphids were dipped in the *S. panduriforme* extracts and two controls (water and malathion) for 5 sec. The leaves were placed into Petri dishes with moist paper towel after allowing them to dry for 5 min, and kept at room temperature of 18 - 21°C. The number of dead aphids in the Petri dishes was recorded after 6, 12 and 24 h.

3.3.3 Aphid dip bioassays

The toxicity for the *S. panduriforme* extracts and two controls (water and malathion) was tested on aphid populations collected from naturally infested field plants. Groups of 10 – 15 aphids were transferred in a tea strainer and dipped in each treatment for 5 sec (Chandrasena *et al.* 2011; Kranthi 2005). The aphids were carefully transferred using a fine camel hair brush from the tea strainer on to rearing leaf cuttings (4 cm x 4 cm) placed in Petri dishes lined with moist paper towel. The aphids in the Petri dishes were classified as dead or alive after 12 h.

3.3.4 Topical bioassays

Acute toxicity of the extracts was determined using topical application desk top bioassays (Sajftrova *et al.* 2013) and measured as aphid mortality. Ten to 15 aphids were tested for each extract and the controls. Leaf cuttings with aphids were placed in Petri dishes lined with moist cotton wool. Each aphid received a droplet of botanical extract dissolved in acetone using a micro syringe. The treated aphids were reared for 12 h at room temperature after which

mortality was assessed. Aphids were considered dead if unable to move when gently prodded and percentage mortality was calculated as:
(Number of dead aphids divided by initial number of aphids) X 100

3.3.5 Choice and no choice bioassays

The bioassays were carried out repetitively using the aphid and leaf dip bioassays. Aphids were reared on treated leaf cuttings and untreated leaf cuttings (Mekuaninte *et al.* 2011). Aphid mortality and number of aphids that moved from treated leaf cuttings (aphid movement) to untreated rearing leaves was recorded after 24 h and 48 h.

3.4 Plant assays or leaf spray bioassays

Field plants and pot plants in the screen house were used for the plant assays (Pavela 2009; Sallam *et al*. 2009; Wabale & Kharde 2010). The young seedlings in pots were artificially infested with aphids and mature field plants were allowed to be naturally infested with the aphids. The plants with high aphid infestation were selected for the plant assays. Leaf sections with aphids were marked to record the number of aphids before spraying. The number of live aphids was recorded after 24 h and 72 h.

3.5 Qualitative phytochemical analysis of aqueous and ethanol extracts from leaves, ripe berries and unripe berries of *S. panduriforme*

The phytochemical analysis tests on aqueous and ethanol extracts was carried out to identify presence of alkaloids, flavonoids, phenolic compounds and saponin believed to be bioactive against aphids. Standard methods were used to examine the reactions from botanical extracts solutions based on colour changes and precipitation (Prashanth & Krishnaiah 2014; Singh & Chauhan 2014). The aqueous and ethanol extraction techniques using maceration and homogenisation procedures were used. Water and 60% ethanol were used as the solvents.

3.6 Data analysis

Toxicity of the extracts was measured and analysed through aphid mortality (Kranthi 2005) after 12 h, 24 h, 36 h, 48 h or 72 h, depending on the assays (bioassays, plant assays or topical bioassays). Effects on aphid behaviour were measured through number of aphids that moved (aphid movement) in choice and no choice bioassays. MSTATC (Nissen 1989) was used for the inferential statistics and Excel (Microsoft Version 2010) was used for the descriptive statistics. MSTATC was also used as necessary to transform the data before analysis.

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CHAPTER 4: EFFECT OF AQUEOUS, ETHANOL, HEXANE AND DIETHYL ETHER EXTRACTS FROM *SOLANUM PANDURIFORME* ON THE CABBAGE APHID (*BREVICORYNE BRASSICAE*).

4.1 Introduction

Botanical insecticides are naturally occurring toxins or extracts derived from secondary plant metabolites (Chowański *et al.* 2016; Singh & Chauhan 2014). They are used by plants to repel insects, to make a plant unpalatable or to attract insects to poison them. Botanical insecticides have become a good option in integrated pest management approaches (Varela *et al*. 2003). To ensure the effectiveness of the botanical insecticides in viable pest management programs, it is important that the factors of production of the botanical insecticides are considered together with innovative application technologies. The factors considered as affecting effectiveness of botanical extracts include extraction and formulation techniques, availability of resources and production costs (Miresmailli & Isman 2014). Extraction and screening bioactive compounds using bioassays and plant assays make possible the identification, isolation and characterization of the effective bioactive components from plant materials (Sasidharan *et al*. 2011; Yang *et al*. 2004). Bioassays and plants assays are used to screen and identify bioactive compounds from various mixtures. It is therefore important to correctly extract and isolate the bioactive compounds for characterisation.

Extraction or separation of the active components from the plant tissues using selective solvents through standard procedures is the first crucial step in identification of bioactive compounds. Extraction procedures help to relocate bioactive constituents from the plant into an extract that can be used to make economic and safer insecticides, medicines and supplements with more stable shelf life than the plants (Handa *et al.* 2008). Extracts are complex, multicomponent mixtures obtained after using a solvent to dissolve components of the botanical material. The choice of solvent is influenced by what the extract is intended for, the targeted compounds or the specific nature of the bioactive compound being targeted (Sasidharan *et al*. 2011; Tiwari *et al.* 2011). The factors considered when choosing a solvent are quantity of phytochemicals to be extracted, rate of extraction, diversity of compounds to be extracted, ease of subsequent handling of the extracts, toxicity of the solvent in the bioassay process and the potential health hazard of the extract (Tiwari *et al.* 2011). Historically, the simplest extracts were made from ethanol and water; water is the universal solvent. Higher amounts of polyphenols are extracted

from ethanol extracts than aqueous extracts and methanol is more polar than ethanol but it is cytotoxic (Tiwari *et al.* 2011; Sasidharan *et al*. 2011). Acetone dissolves many hydrophilic and lipophilic components. If the desired active constituents are glycosides, polar solvents like aqueous methanol may be used (Handa *et al*. 2008). Tannins and terpenoids are more often obtained by treatment with less polar solvents. It is therefore important to use a solvent that extracts the desired chemical components from the plant materials for further separation and characterisation and possibly synthesis into a product for use (Sasidharan *et al*. 2011; Sajfrtova *et al.* 2013). Some of the active components present in extracts from the various solvents are as shown in Table 4.1.

Table 4.1: Active components in extracts from different solvents

Solvent	**Active Components**
Water	Tannins, Saponins, Terpenoids
Ethanol	Tannins, polyphenols, flavonol, alkaloids
Methanol	Saponins, Taninns, polyphenols,
Chloroform	Flavonoids
Ether	Alkaloids
Acetone	Phenols, flavonoids

Adapted from Tiwari *et al.* (2011)

The solvents to use may extract a wide range of bioactive compounds or may be chosen for a more selective action. Ethanol has different properties than water and can extract different bioactive compounds than water. A mix of water and alcohol is generally better at extracting a wider variety of constituents than either one alone (Tiwari *et al*. 2011). When mixtures of alcohol and water of up to 60% – 70% alcohol were used, there was improved effectiveness of the extracts for medicinal use when compared with pure compounds (Tiwari *et al.* 2011; Sasidharan *et al*. 2011). The ratio between water and alcohol is varied to suit the particular plant being extracted. The choice of solvent helps to determine exactly what and how much gets extracted from the plant into the extract.

A number of extraction techniques or methods have been used, their effectiveness, advantages and disadvantages have been explained mainly for the medical and pharmaceutical purposes. The extraction methods include, among others, maceration extraction, homogenisation extraction, solvent assisted/sequential/ successive extraction and decoction extraction (Handa

et al. 2008; Tiwari *et al.* 2011; Yang *et al*. 2004). The effectiveness, quality and quantity of bioactive compounds from plant extracts is not necessarily determined by the extraction method used but by a number of factors which include solvent type and concentration, extraction temperature, time for extraction and the volume of solvent to plant material (Sasidharan *et. a*l 2011; Yang *et al*. 2004).

The temperature to use for extraction depends on the solvent and the extraction method. Soxhlet, maceration and homogenisation extraction may be carried out at room temperature (Ntonifor *et al*. 2010; Sasidharan *et al*. 2011). However hot water extraction has been reported to be better than cold water extraction. Katsvanga *et al.* (2006) reported a higher cabbage aphid mortality from extracts collected at temperature of 60 °C than 50 °C and 65 ^{0}C using the homogenization method. Other researchers have extracted effective plant extracts using maceration or Soxhlet extraction at temperature of 60 °C – 80 °C (Singh & Chaudhan 2014; Srivastava & Guleria 2003). For solvent assisted/ sequential extraction technique when the residue of the previous extraction is used as the feed for the next extraction, the extraction temperature used by Yang *et al*. (2004) was 70 °C for hexane, chloroform and ethyl acetate, 85 °C for methanol, 100 °C for methanol/water, and 120 °C for water. Extraction at 90 °C showed an increased phenolics content (Azwanida 2015), which are active against aphids.

The time required for extraction ranges from three hours for Soxhlet extraction to four days for maceration extraction. Sajfrotova *et al*. (2013) used Soxhlet extraction using hexane and ethanol as the solvents to extract bioactive compounds from thyme and savory for 7 h; the extracts were effective against the larvae of Colorado potato beetle. Other researchers used 24 h – 48 h for homogenisation and 48 h – 96 h for maceration (Ntonifor *et al.* 2010; Wabale & Kharde 2010). The sample to solvent ratio generally used in most studies is 1:10 w/v for dried material (Tiwari *et al.* 2011), though 1:25 w/v, 1:15 w/v; 1:5 w/v and 1:2 w/v have been used (Ntonifor *et al*. 2010; Sajfrotova *et al*. 2013; Srivastava & Guleria 2003). For fresh extracts, the ratios that were used to extract fresh neem and eucalyptus leaf extracts to control aphids were 1:1 w/v and 1:2 w/v with or without further dilution (Bahar *et al*. 2007; Gonzalez *et al*. 2011; Wabale & Kharde 2010). Commercial botanical extracts (emulsions and essential oils) have also been used to test for effectiveness of botanicals against aphids, leaf hoppers and white flies (Amoabeng *et al*. 2013; Pavela 2009; Ranger *et al*. 2009).

Maceration and homogenisation have been suggested as more applicable, convenient and less costly methods for small and medium extraction compared to more complex extraction methods like Soxhlet and high performance liquid chromatography (HPLC) (Azwanida 2015). Due to high cost associated with other methods and despite their efficiency to extract bioactive compounds, they are rarely used at small to medium scale extraction, especially in the extraction of medicinal plants. However, all the factors (temperature, solvents, time of extraction and plant material to solvent ratio) that influence composition and effectiveness of extracts have the ability to enhance extraction, proper information about the factors is required to avoid degradation of the bioactive compounds. It is suggested and important to consider methods that have least influential factors as suitable methods of extraction for botanical insecticides in order to achieve maximum effectiveness of the extract (Azwanida 2015; Tiwari *et al.* 2013).

To find and describe new insecticides derived from natural products, routine research and extraction of bioactive compounds and testing of the botanical extracts is important (Chowański *et al.* 2016). Extracts must be screened several times using technologically simple, inexpensive and rapid bioassays to determine their bio-effectiveness against the insect pests (McLaughlin *et al*. 1998). Bioassays play an important role in screening the extracts and they have a special advantage in the standardisation of botanical products (CIPS 2007; McLaughlin *et al.* 1998). Bioassays play a key role in identifying the bio-effects of pesticidal plants for subsequent analysis with liquid chromatography or spectrometry to confirm if the bioactivities are due to desired compounds (McLaughlin *et al*. 1998; Sasidharan *et al*. 2011). Separation techniques using chromatography and structural elucidation methods can only be employed after identifying the bio-effects of pesticidal plants (Sasidharan *et al.* 2011), thus repetitive assays (bioassays and plant assays) are used to screen the bioactive botanicals before they are accepted and incorporated into legitimate long term practices (Chowański *et al.* 2016; McLaughlin *et al.*1998; Stell *et al.* 2013).

A number of bioassay techniques are available for screening effectiveness of plant extracts against insects. The bioassays include leaf dip/leaf residual/leaf disc bioassays for sap sucking and leaf eating insects, foliar application bioassays for Lepidoptera larvae, oral feeding for bollworms and sap sucking insects, topical application bioassays for Lepidoptera and Homoptera insects that are large enough to take 1 µml of insecticide, thin layer exposure bioassay/surface residue vial bioassays, sticky card technique bioassays for white flies and thrips, slide dip bioassays for small bodied insects and young larvae, glass vial/ vial residue

bioassays for Lepidoptera larvae and larval dip/aphid dip bioassays for larvae and soft bodied insects (CIPS 2007; Kranthi 2005). Toxicity of botanical formulations on soil insects such as white grubs and the wire worms has also been measured using *in vitro* soil dip bioassays (Ranger *et al*. 2009). Diet incorporation assays for oral feeding may also be used to assess effect of toxins on oral feeding in Lepidoptera larvae (Pavela 2009).The choice of bioassays to use depends on operator skills, materials availability and handling procedures, and they may be modified to suit the practical conditions (CIPS 2007; Kranthi 2005). The topical bioassay, leaf disc residual, oral feeding, topical application and vial residual bioassays have been more commonly used over the past two decades, depending on the insect of interest (CIPS 2007; Kranthi 2005). Field collected or artificially reared insects are used as test insects and most bioassays response to insecticides effectiveness rely on mortality as the response to toxicity (CIPS 2007).

According to McLaughlin *et al.* (1998) and Stell *et al*. (2013), for bioactive botanicals to be accepted and incorporated into legitimate long term practices, extracts must be screened and analysed many times over and over again after pre-screening with replication on different dates. The current research was set out to determine the effect of plant material, and extraction thereof, on mortality of the cabbage aphid. Extracts from leaf powder, berry powder, fresh ripe berries and fresh unripe berries of *Solanum panduriforme* were extracted and tested on the cabbage aphid, *Brevicoryne brassicae.*

4.2 Aim and objectives

The aim of this research was to investigate the effects of aqueous, ethanol and solvent assisted extracts of *Solanum panduriforme* from young leaves, ripe berries and unripe berries on the mortality of the cabbage aphid using bioassays.

The objectives were:

1. To investigate the effects of three concentrations of aqueous and ethanol extracts of *S. panduriforme* on the cabbage aphid, *Brevicoryne brassicae,* using three desk top bioassay techniques.
2. To evaluate the effects of aqueous extracts of *S. panduriforme* on the behaviour of *B. brassicae* using choice and no choice bioassays.
3. To evaluate the effectiveness of solvent assisted (hexane and diethyl ether) dried crude extracts on the mortality of cabbage aphids *B. brassicae* using topical application bioassays.

4.3 Materials and methods

4.3.1 Extraction methods and procedures

4.3.1.1. The plant materials

The plant materials used were dried young leaves (leaf powder), dried ripe berries (berry powder), fresh ripe berries and fresh unripe berries of *S. panduriforme*, collected from an abandoned field at the University of Venda in Limpopo Province (22.9761° S, 30.4465° E), South Africa.

4.3.1.2 Extraction procedures

The extraction procedures used for aqueous and ethanol extracts were plant tissue homogenisation for the powders and maceration for the fresh berries (Chiffele *et al.* 2009; Tiwari *et al.* 2011). Homogenisation involved soaking the powders in the solvents for 24 h and maceration involved soaking the fresh berries in the solvents for 48 h with frequent agitation to dissolve soluble matter. The plant materials were mixed with the two solvents separately; distilled water to produce aqueous extracts and 60% ethanol to produce ethanol extracts. To produce aqueous extracts from the powders, 50 g of plant material per 500 ml (1:10 w/v) was initially used but the extract was almost a paste (Fig. 4.1a) indicating very high powder content. It was not possible to use the paste mixture used to carry out the bioassays; the mixture was diluted with 500 ml distilled water to make it 50 g per 1000 ml ending up with 1:20 w/v. This became the stock solution (1:20 w/v) which was filtered through a Whatman filter paper (number 9). The stock solution was diluted to 50%, 25% and 12.5% (1:40w/v, 1:80w/v and 1:160w/v) and used for the bioassays (Fig.4.1b). To produce aqueous extracts from the fresh berries, half sliced fresh ripe berries and unripe berries at 250 g berries per 500 ml were placed in a tightly sealed container with frequent agitation to dissolve the soluble matter. The mixture was filtered through a mutton cloth to obtain a stock solution (1:2 w/v). The stock solution was diluted to 50%, 25% and 12.5% (1: 4 w/v, 1:8 w/v and 1: 16 w/v) for use in the bioassays.

To produce ethanol extracts from powders, 40 g powder in 500 ml 60% ethanol was used. The mixture was also a paste; it was diluted with 500 ml distilled water before filtering through a Whatman filter paper (number 9). To produce ethanol extracts from fresh berries, half sliced fresh ripe berries and unripe berries at 250 g per 500 ml were placed in tightly sealed containers with frequent agitation. The stock solution was further diluted with distilled water to 50%, 25% and 12.5% before using in bioassays.

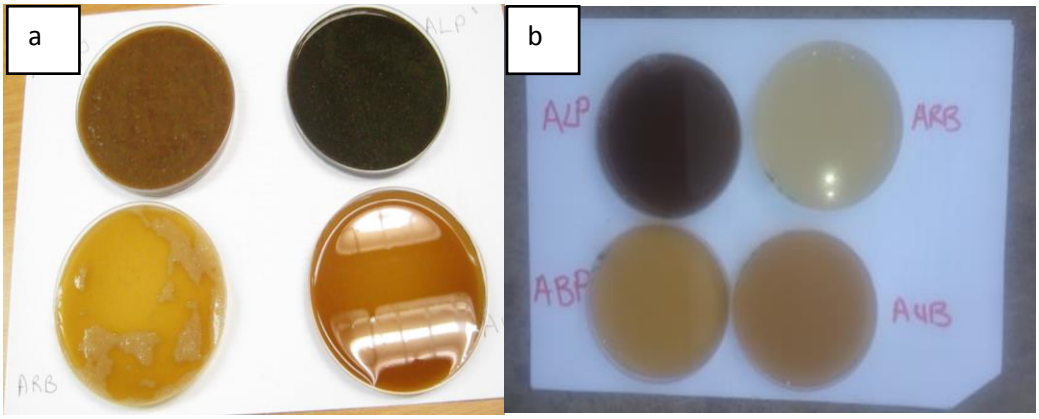

Figure 4.1 Aqueous extracts

a) Powders 1:10 w/v (top) and fresh berries 1:2 w/v (bottom)
b) Powders 1:40 w/v (left) and fresh berries 1:4 w/v (right)

The solvent-assisted sequential extraction method using simple fractionation was used to extract polar and non-polar compounds from leaf powder, berry powder, fresh ripe berries and fresh unripe berries of *S. panduriforme* using water, hexane and diethyl ether (Jeyaseelan 2012). The aqueous extraction rates of 10 g per 400 ml for powders and 100 g per 400 ml for fresh berries were used. The initial aqueous extracts were filtered using pressurised water. The same aqueous extract was then mixed with 400 ml hexane and later with 400 ml diethyl ether extracts to isolate the polar and non-polar compounds. The hexane and diethyl ether extracts were air-dried in a fume cabinet, weighed and kept in vials in the refrigerator until used in topical bioassays.

Unscented liquid dishwasher was added to each extract mixture at 1 ml per litre as a wetting agent to ensure effective coating of the leaf or aphid surface, depending on the bioassays carried out.

4.3.2: Laboratory bioassays

Preliminary bioassays were carried out to pre-screen and test the effect of three concentration levels of aqueous and ethanol extracts on the cabbage aphid mortality. Subsequent bioassays were repeatedly carried out to test the effectiveness of one concentration level on the mortality of the cabbage aphid using three different bioassays. No choice and choice bioassays were used to test the effects of aqueous extracts on the behaviour of the aphids. Desk top bioassays (McLaughlin *et al.* 1998) were used to determine the insecticidal effect of *S. panduriforme* on the cabbage aphid *B*. *brassicae*. These were repeatedly carried out using preliminary bioassays

followed by subsequent bioassays, no choice and choice bioassays and finally topical bioassays. Three brassica species (*B. olaracea, B. napus* and *B. juncea*) were used to carry out the bioassays. The aqueous and ethanol extracts were tested for their effectiveness against the cabbage aphid, by carrying out initial preliminary bioassays on the effectiveness of extract concentration on the cabbage aphid mortality using leaf dip residual bioassays. The effective concentration from the preliminary bioassays was then tested against the cabbage aphid mortality using three different bioassays (leaf dip residual, aphid and leaf dip and aphid dip bioassays). Aphid mortality was assessed for the three bioassays. The effects of aqueous extracts on behaviour of aphids were also evaluated in choice and no choice bioassays on treated and untreated rearing leaves. The solvent-assisted sequentially extracted dried crude extracts from hexane and diethyl ether were tested for toxicity against the cabbage aphid assessed as aphid mortality using topical bioassays.

4.3.2.1: Preliminary bioassays to test the effect of three concentration levels of *S. panduriforme* on the cabbage aphid mortality

The effect of aqueous and ethanol extracts of *S. panduriforme* from leaf powder, berry powder, fresh ripe berries and fresh unripe berries at three concentration levels was tested against the cabbage aphid *B. brassicae* mortality using leaf dip/disc or leaf residual bioassays. Fresh young leaves were collected from mustard/Indian rape (*B. juncea*) field plants at the University of Venda. Leaf squares (4 cm x 4 cm) cut from the mustard rape leaves were dipped for 10 sec separately in 24 *S. panduriforme* extracts and the water control as shown in Table 4.2 (Munir & Muhammad 2005; Moslemi *et al.* 2011; Sallam *et al.* 2009). The leaf squares were air dried for 5 min after dipping in the extracts and placed separately upside down in a Petri dish lined with moist paper towel to reduce desiccation (Fig. 4.2a). Ten to 15 aphids from a field population (Fig. 4.2b) were transferred to each leaf square by means of a camel-hair brush (Fig. 4.2c). The completely randomised design (CRD) with 25 treatments and three replications was used. The number of dead aphids in each Petri dish was recorded at 12, 24 and 36 h after transferring the aphids. Aphids that did not move after a gentle touch were considered dead. The 25 preliminary bioassays treatments were from 12 aqueous extracts, 12 ethanol extracts and 1 control of water alone.

The 12 aqueous extracts treatments were:

1) Three concentrations from leaf powder (1:40 w/v, 1:80 w/v and 1:160 w/v from an original stock solution of 1:20 w/v)
2) Three concentrations from ripe berries powder (1:40 w/v, 1:80 w/v and 1:160 w/v from an original stock solution of 1:20 w/v)
3) Three concentrations from sliced fresh ripe berries (1:4 w/v, 1:8 w/v and 1:16 w/v from an original stock solution of 1:2 w/v)
4) Three concentrations from sliced unripe fresh berries (1:4 w/v, 1:8 w/v and 1:16 w/v from an original stock solution of 1:2 w/v).

The 12 ethanol extracts treatments were:

1) Three concentrations from leaf powder (1:50 w/v, 1:100 w/v and 1:200 w/ from an original stock solution of 1:25 w/v),
2) Three concentrations from ripe berries powder (1:50 w/v, 100 w/v and 1:200 w/v from an original stock solution of 1:25 w/v)),
3) Three concentrations from sliced fresh ripe berries (1:4 w/v, 1:8 w/v and 1:16 w/v, from an original stock solution was 1:2 w/v)
4) Three concentrations from sliced fresh unripe berries (1:4 w/v, 1:8 w/v and 1:16 w/v from an original stock solution was 1:2 w/v).

Table 4.2: The four aqueous and four ethanol extracts at three concentration levels used as the treatments for the preliminary bioassays designed as 8 treatments x 3 levels x 3 replications factorial experiment in a completely randomised design

Plot	Treatment	Extract level	Concentration
1	Aqueous leaf powder (ALP)	ALP high	1:40 w/v
2		ALP medium	1:80 w/v
3		ALP low	1:160 w/v
4	Aqueous berry powder (ABP)	ABP high	I:40 w/v
5		ABP medium	1:80 w/v
6		ABP low	1:160 w/v
7	Aqueous ripe berries (ARB)	ARB high	1:4 w/v
8		ARB medium	1:8 w/v
9		ARB low	1:16 w/v
10	Aqueous unripe berries (AUB)	AUB high	1:40 w/v
11		AUB medium	1:80 w/v
12		AUB low	1:160 w/v
13	Ethanol leaf powder (ELP)	ELP high	1:50 w/v
14		ELP medium	1:100 w/v
15		ELP low	1:200 w/v
16	Ethanol berry powder (EBP)	EBP high	1:50 w/v
17		EBP medium	1:100 w/v
18		EBP low	1:200 w/v
18	Ethanol ripe berries (ERB)	ERB high	1:4 w/v
20		ERB medium	1:8 w/v
21		ERB low	1:16 w/v
22	Ethanol unripe berries (EUB)	EUB high	1:4 w/v
23		EUB medium	1:8 w/v
24		EUB low	1:16 w/v
25	Control	Water	Distilled

Figure 4.2: The preliminary bioassays methods

a) Leaf cuttings in petri dishes lined with moist paper towel for desk top bioassays
b) Field aphids used in the bioassays from mustard rape plants collected from field
c) Transferring aphids onto leaf cuttings using a camel-hair brush

4.3.2.2: Subsequent bioassays to test the effect of one concentration from the preliminary bioassays using three different bioassays

The effect of one most effective concentration level from ethanol and aqueous extracts determined from the preliminary bioassays was tested in subsequent bioassays using three different bioassay techniques (leaf dip residual, leaf and aphid dip and aphid dip). The concentration levels used were 1:40 w/v in water and 1:50 w/v in 60% ethanol for powders and 1:4 w/v for fresh ripe and fresh unripe berries for both water and 60% ethanol. The subsequent bioassays treatments were based on preliminary bioassays results which showed that the highest concentration was consistently most effective. There were 10 treatments (Table 4.3) made up of four aqueous extracts (leaf powder, berry powder, fresh ripe berries and fresh

unripe berries), four ethanol extracts (leaf powder, berry powder, fresh ripe berries and fresh unripe berries) and two controls (water and malathion). The CRD design with 10 treatments and three replications was used. The three bioassays techniques used were the leaf dip residual bioassays; aphid and leaf dip bioassays and the aphid dip bioassays.

Table 4.3: The aqueous and ethanol extracts at one concentration level used as treatments for the leaf dip residual, aphid and leaf dip and aphid dip subsequent bioassays

Treatment	Extract	Concentration
1	Aqueous leaf powder (ALP)	1:40 w/v
2	Aqueous berry powder (ABP)	1:40 w/v
3	Aqueous ripe berries (ARB)	1:4 w/v
4	Aqueous unripe berries (AUB)	1:4 w/v
5	Ethanol leaf powder (ELP)	1:50 w/v
6	Ethanol berry powder (EBP)	1:50 w/v
7	Ethanol ripe berries (ERB)	1:4 w/v
8	Ethanol unripe berries (EUB)	1:4 w/v
9	Malathion 50% EC	7ml / 5L water
10	Water	Distilled

4.3.2.2:1 Leaf dip or leaf cuttings residual bioassays

Mustard rape leaf squares (4 cm x 4 cm) were separately dipped in 10 treatments (eight *S. panduriforme* extracts and two controls, water and malathion) for 10 sec (Munir & Muhammad 2005; Moslemi *et al.* 2011; Sallam *et al.* 2009). The cuttings were air dried for 5 min before each leaf cutting was placed separately upside down in a petri dish lined with moist kitchen paper to reduce desiccation. Ten to 15 aphids were released on each leaf cutting by means of a camel-hair brush. The number of dead aphids from each petri dish was recorded 12, 24 and 36 h after releasing the aphids. Aphids which did not move after a gentle touch were considered dead.

4.3.2.2.2: Aphid and leaf dip bioassays

Detached cabbage (*Brassica oleracea*) leaf cuttings from field plants with naturally infested aphids (Fig. 4.3a) were used (Atteyat *et al*. 2012; Yi *et al.* 2012). The leaf cuttings were checked under a microscope to ensure that approximately 20 similar wingless adult aphids remained on each leaf cutting. The leaf cuttings with the aphids were dipped separately in 10 treatments (eight *S. panduriforme* extracts and two controls, water and malathion) for 5 sec. The leaves were placed into Petri dishes with moist paper towel after allowing them to dry for 5 min, and

kept at room temperature range of 18 - 21 °C. The number of dead aphids in the Petri dishes was recorded after 6, 12 and 24 h.

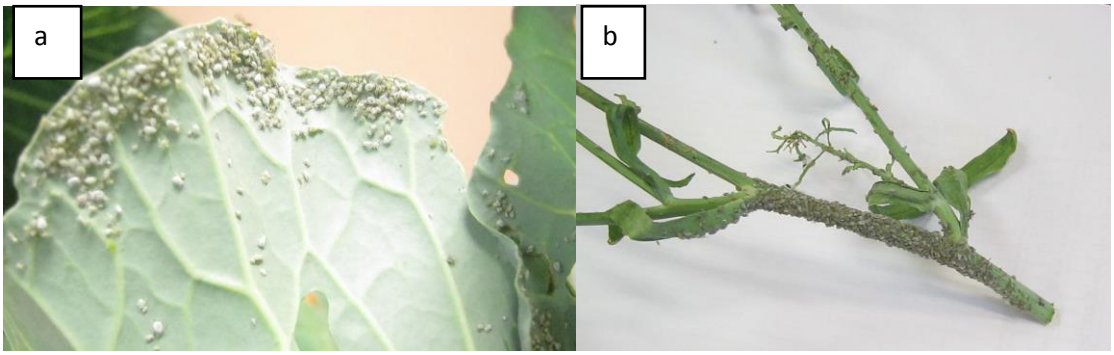

Figure 4.3: Aphids used for the bioassays

a) Aphids on cabbage leaves from the field used for aphid and leaf dip bioassays
b) Aphids on a flowering mustard rape plant used for aphid dip bioassays

4.3.2.2.3: Aphid dip bioassays

The toxicity for the eight *S. panduriforme* extracts and two controls (water and malathion) was tested on aphid populations collected from field naturally infested mustard rape plants (Fig.4.3b). Groups of 10 – 15 aphids were transferred in a tea strainer and dipped in each treatment for 5 sec (Chandrasena *et al.* 2011; Kranthi 2005). The aphids were carefully transferred using a fine camel hair brush from the tea strainer on to rearing mustard rape leaf cuttings (4 cm x 4 cm) which were placed in Petri dishes lined with moist paper towel. The aphids in the Petri dishes were classified as dead or alive after 12 h.

4.3.2.3: The effect *S. panduriforme* aqueous extracts on the cabbage aphid in choice and no choice bioassays

The effect of *S. panduriforme* aqueous extracts on the behaviour of aphids was evaluated in choice and no choice aphid and leaf dip bioassays using *B. napus* leaves. The CRD design with six treatments (Table 4.4) replicated three times was used. The bioassays were carried out repetitively using treated rearing leaf cuttings and untreated rearing leaf cuttings which provided a choice and no choice scenario for the aphids.

Table 4.4: The aqueous extracts used for choice and no choice bioassays

Treatment	Extract	Concentration
1	Aqueous leaf powder (ALP)	1:40 w/v
2	Aqueous berry powder (ABP)	1:40 w/v
3	Aqueous ripe berries (ARB)	1:4 w/v
4	Aqueous unripe berries (AUB)	1:4 w/v
5	Malathion 50% EC	7 ml / 5 l water
6	Water	Distilled

4.3.2.3.1: Choice bioassays on untreated rearing leaf cuttings

The treated aphids from the aphid and leaf dip cuttings (Fig 4.4) were reared on untreated rearing leaf cuttings which served as an alternative food source for the aphids (Bandeira *et al.* 2012; Mekuaninte *et al.* 2011). Aphid survival was recorded after 24 h and 48 h. Observations on aphid behaviour (disturbed settling and movement) after treatment and during mortality assessments were also recorded.

Figure 4.4: Leaf and aphid dip bioassays treated with aqueous extracts and placed on untreated leaf cuttings for the choice bioassays.

4.3.2.3.2 No choice bioassays on treated rearing leaf cuttings

In the no choice test adapted from Sajfrtova *et al.* (2013), the treated aphids from the aphid and leaf dip cuttings were reared on treated rearing leaf cuttings which were also dipped in the same extract as the aphid and leaf dip cutting (Fig. 4.5 and Fig. 4.6). Aphid survival was recorded at 24 h, 48 h and 72 h after dipping in the extracts. Observations on aphid behaviour (disturbed settling and movement) after treatment and during mortality assessments were also recorded.

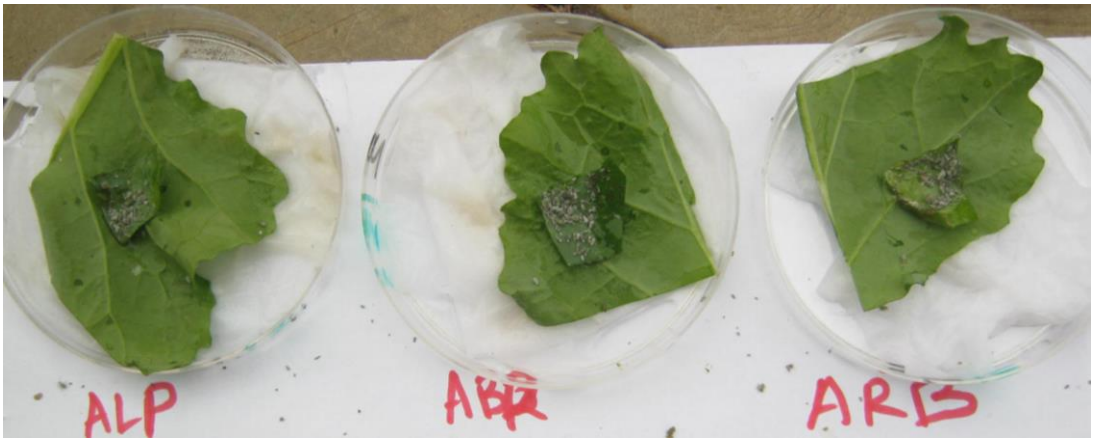

Figure 4.5: Leaf and aphid dip bioassays treated with aqueous extracts on treated rearing leaves (Left – Right: aqueous leaf powder, aqueous berry powder and aqueous ripe berries)

Figure 4.6: Leaf and aphid dip bioassays treated with aqueous extracts on treated rearing leaves (Left – right: aqueous unripe berries, malathion and water)

4.3.2.4: The effect of solvent assisted (hexane and diethyl ether) extracts from *S. panduriforme* on the cabbage aphid

Dried crude extracts from hexane and diethyl ether were dissolved in acetone (Abbad & Beshel 2013; Ntonifor *et al.* 2010) at 50 mg /ml (Coehlo *et al*. 2006). There were 14 treatments (Table 4.5) from three successive extractions (water, hexane, diethyl ether) from leaf powder, berry powder, fresh ripe berries and fresh unripe berries; acetone alone and distilled water were used as controls. The CRD design with 14 treatments replicated three times was used. Acute toxicity of the extracts was determined through topical application on the aphids from desk top bioassays (Sajftrova *et al.* 2013) and measured as aphid mortality. Ten - 15 aphids were tested for each extract and the controls. Leaf cuttings with aphids were placed in Petri dishes lined with moist cotton wool (Fig. 4.7). Each aphid received a droplet of acetone botanical extract solution using a micro syringe. The treated aphids were reared for 24 h at room temperature after which mortality was assessed. Aphids were considered dead if unable to move when gently prodded and percentage mortality was calculated.

Table 4.5: Treatments used in the topical bioassays based on solvents used for the solvent assisted extraction method

Treatment	Description
1	Leaf powder hexane extract (LPH)
2	Leaf powder diethyl ether extract (LPEE)
3	Leaf powder aqueous extract (ALP)
4	Berry powder hexane extract (BPH)
5	Berry powder diethyl ether extract (BPEE)
6	Berry powder aqueous extract (ABP)
7	Fresh ripe berries hexane extract (RBH)
8	Fresh ripe berries diethyl ether extract (RBEE)
9	Fresh ripe berries aqueous extract (ARB)
10	Fresh unripe berries hexane extract (UBH)
11	Fresh unripe berries diethyl ether extract (UBEE)
12	Fresh unripe berries aqueous extracts (AUB)
13	Acetone (control)
14	Water (control)

Figure 4.7: Aphids on leaf cuttings for topical application bioassays

4.4 Results and discussion

Data were analysed using MSTATC (Nissen 1989). Graphs were created using Excel (Microsoft Version 2010). The results from all the bioassays showed that the leaves and berries of *S. panduriforme* have varied insecticidal effects on the cabbage aphid, depending on the solvent used for extraction, plant part and bioassay carried out. The aphid mortality data for the preliminary bioassays (effect of concentration), subsequent bioassays (effect of ethanol and aqueous extracts), choice and no choice bioassays (effect of aqueous extracts on aphid behaviour) and topical bioassays (effect of solvent dried crude extracts) are shown and discussed below.

4.4.1 Effect of *S. panduriforme* extract concentration on aphid mortality

4.4.1.1 Preliminary bioassays results

The mortality of aphids from the preliminary leaf dip leaf residual bioassays ranged from 7% for water extracts to 100% for ethanol ripe berries extracts. The means for mortality after 24 h from the different extract solutions for the three levels of concentrations are shown in Table 4.6. There were significant differences in aphid mortality among the botanical extracts concentrations ($P< 0.05$). Total mortalities (100%) were from ethanol extracted ripe and unripe berries at the highest concentration, but these did not differ significantly from ethanol extracted leaf powder and berry powders at all concentrations; however ethanol extracted ripe berries at the lowest concentration was significantly different from leaf powder and berry powder. Aqueous unripe berries extract at highest concentrations was also not significantly different from the ethanol extracts at 90% mortality; this was the highest mortality from aqueous extracts which was significantly different from all other aqueous extracts. Mortalities from 70% to 100% did not

differ significantly from each other (Table 4.6), There was more variation in mortality from aqueous extracts among the concentrations and among the plant parts. However the highest concentrations from aqueous extracts were technically effective with above 70 % aphid mortality similar to the highest concentrations from the ethanol extracts (Fig. 4.7). There were no significant differences in mortality from the ethanol concentrations except the ripe berries, as mentioned before.

The highest concentration generally had higher mortality and the lowest concentration generally had lower mortality. The aphid mortality was generally higher from 1:40 w/v for powders and 1:4 w/v for fresh berries and generally lower from 1: 160 w/v for powders and 1:16 w/v for fresh berries for both aqueous extracts and alcohol extracts (63% versus 49% for aqueous leaf powder, 64% versus 52% for aqueous berry powder, and 90% versus 71% for aqueous unripe berries; 96% and 75% for ethanol leaf powder, 96% versus 94% ethanol berry powder, 100% versus 63% ethanol ripe berries, 100% and 81% ethanol unripe berries). Aqueous fresh ripe berries mortality was higher for the lowest concentration than the highest concentration (84% versus 65%) even though they were all dipped in the extracts for 5 sec. This deviation could not be explained. The effects of the ethanol extracts are shown in Fig. 4.8a, with dead aphids from ethanol ripe berries compared to the surviving aphids from water control in Fig. 4.8b. The aphids from water treated leaves (control) showed a lighter waxy colour and appeared lively and healthy after 36 h.

Some aphids from aqueous and ethanol extracts (young leaves, ripe berries and unripe berries) of *S. panduriforme* showed a darker colour and had no movement after being prodded or touched, an indication they were dead; some aphids had the cabbage aphid waxy green colour with some movements when lightly touched during assessments, indicating they survived the treatments. New nymphs were observed from the water control and the lowest concentrations of aqueous berry powder and aqueous unripe berries. The surviving aphids were able to reproduce on the water control and the lowest concentrations of the fresh ripe berries extracts. The surviving aphids on the leaves treated with the lowest concentration of aqueous leaf powder also formed colonies after 36 h; an indication that the aphids behaviour was not affected since they managed to settle and formed colonies.

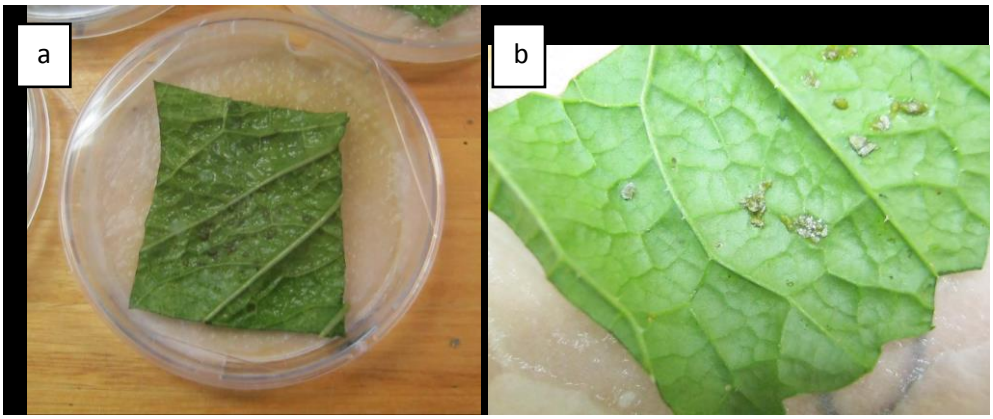

Figure 4.8: Treated aphids on leaf dip residual bioassays after 24h

a) Ethanol leaf powder treatment showing dead aphids
b) Water control showing live aphids

Table 4.6: The mean mortality from preliminary bench top bioassays after 24 h

Plant Extract Treatment	Concentration level	Dilution rate	Aphid mortality (%)
Ethanol Ripe Berries (ERB)	Highest	1:4 w/v	100.0 a
Ethanol Unripe Berries (EUB)	Highest	1:4 w/v	100.0 a
Ethanol Leaf Powder (ELP)	Highest	1:50 w/v	96.7 ab
Ethanol Berry Powder (EBP)	Highest	1:50 w/v	96.3 ab
Ethanol Berry Powder (EBP)	Medium	1:100 w/v	95.8 ab
Ethanol Berry Powder (EBP)	Lowest	1:160 w/v	94.4 ab
Ethanol Ripe Berries (ERB)	Medium	1:8 w/v	94.4 ab
Aqueous Unripe Berries (AUB)	Highest	I:40 w/v	90.3 abc
Ethanol Leaf Powder (ELP)	Medium	1:100 w/v	89.7 abcd
Ethanol Unripe Berries (EUB)	Medium	1:8 w/v	87.4 abcde
Aqueous Ripe Berries (ARB)	Lowest	1:16 w/v	84.3 abcde
Ethanol Unripe Berries (EUB)	Lowest	1:16 w/v	81.2 abcde
Ethanol Leaf Powder (ELP)	Lowest	1:200 w/v	75.9 abcdef
Aqueous Unripe Berries (AUB)	Medium	1:80 w/v	73.9 bcdefg
Aqueous Ripe Berries (ARB)	Medium	1:80 w/v	72.6 bcdefg
Aqueous Ripe Berries (ARB)	Medium	1:8 w/v	72.6 bcdefg
Aqueous Unripe Berries (AUB)	Lowest	1:16 w/v	71.9 bcdefg
Aqueous Leaf Powder (ALP)	Highest	1:40 w/v	68.3 cdefg
Aqueous Berry Powder (ABP)	Medium	I:40 w/v	68.3 cdefg
Aqueous Ripe Berries (ARB)	Highest	I:4 w/v	64.5 defg
Aqueous Berry Powder (ABP)	Highest	I:40 w/v	64.3 defg
Ethanol Ripe Berries (ERB)	Lowest	1:16 w/v	63.9 efg
Aqueous Berry Powder (ABP)	Lowest	I:40 w/v	52.6 fg
Aqueous Leaf Powder (ALP)	Medium	1:80 w/v	52.2 fg
Aqueous Leaf Powder (ALP)	Lowest	1:160 w/v	49.6 g
Water	Distilled	Distilled	7.0 h

LSD at $P < 0.05$: 25.7 CV: 20.1%

Treatments with the same letter are not significantly different

The aphid mortality generally increased from 12 h up to 36 h, as shown in Fig.4.9 (12 h), Fig. 4.10 (24 h) and Fig 4.11 (36 h), indicating residual toxic effects of the extracts on the aphids. After 36 h, the ethanol extracts were generally more effective than the aqueous extracts at the highest concentration and the aqueous fresh unripe berries generally more effective than the powders and the fresh ripe berries. The powders were generally less effective than the fresh berries. After 36 h the aphids in the water control survived but the aphids on the malathion control were dead; aphid survival was highest from the water control treated leaf cuttings as indicated by a low mortality of 7%.

Table 4.7: Means of mortality for highest concentrations after 36 h

Treatment	**Aphid mortality (%)**
Ethanol fresh ripe berries	100
Ethanol uripe berries	100
Ethanol leaf powder	97
Ethanol berry powder	96
Aqueous unripe berries	94
Aqueous ripe berries	87
Aqueous berry powder	78
Aqueous leaf powder	71
Distilled water	7

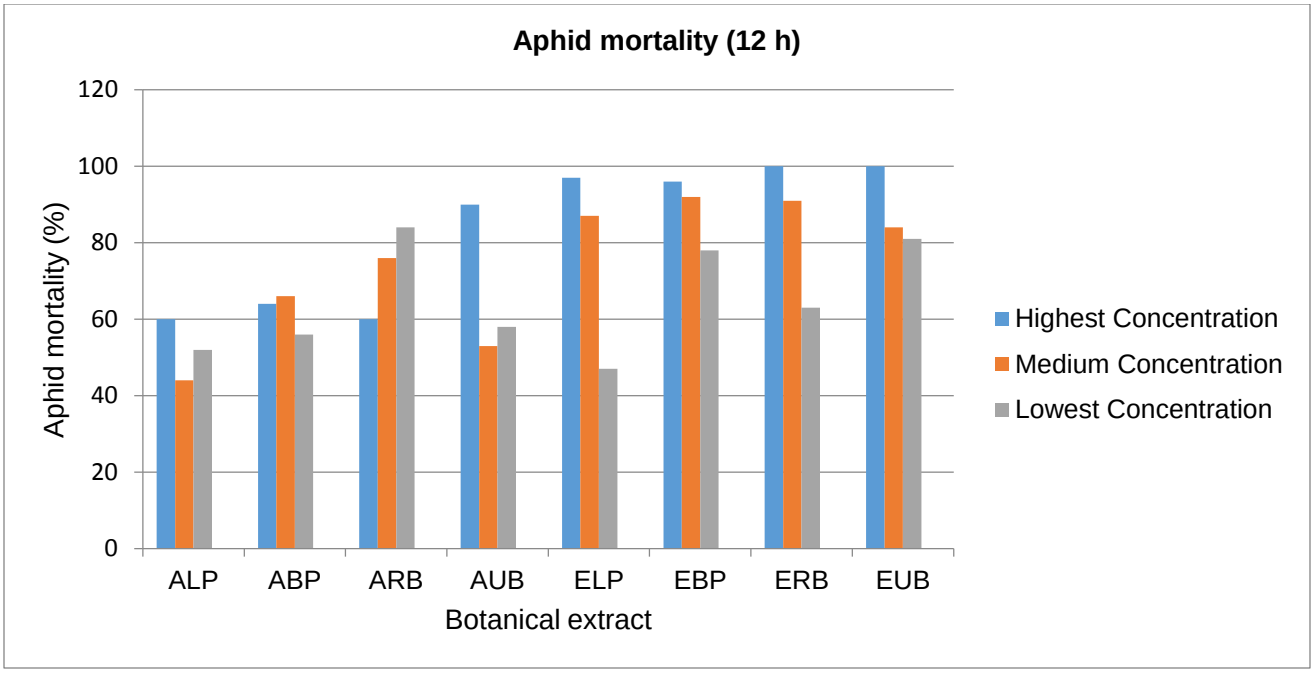

Figure 4.9: Aphid mortality after 12 h from aqueous and ethanol extracts of *S. panduriforme* at three concentration levels.

ALP is aqueous leaf powder, ABP is aqueous berry powder, ARB is aqueous fresh ripe berries, AUB is aqueous fresh unripe berries, ELP is ethanol leaf powder, ERB is ethanol berry powder, and EUB is ethanol unripe berries

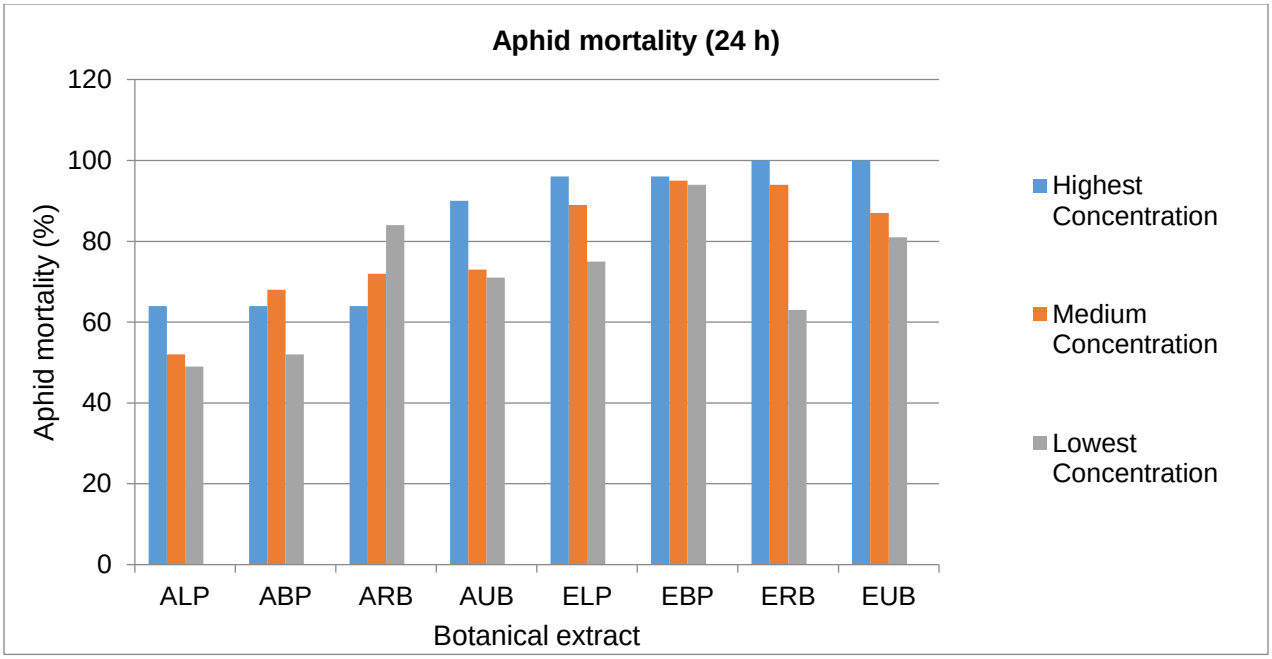

Figure 4.10: Aphid mortality after 24 h from aqueous and ethanol extracts of *S. panduriforme* at three concentration levels

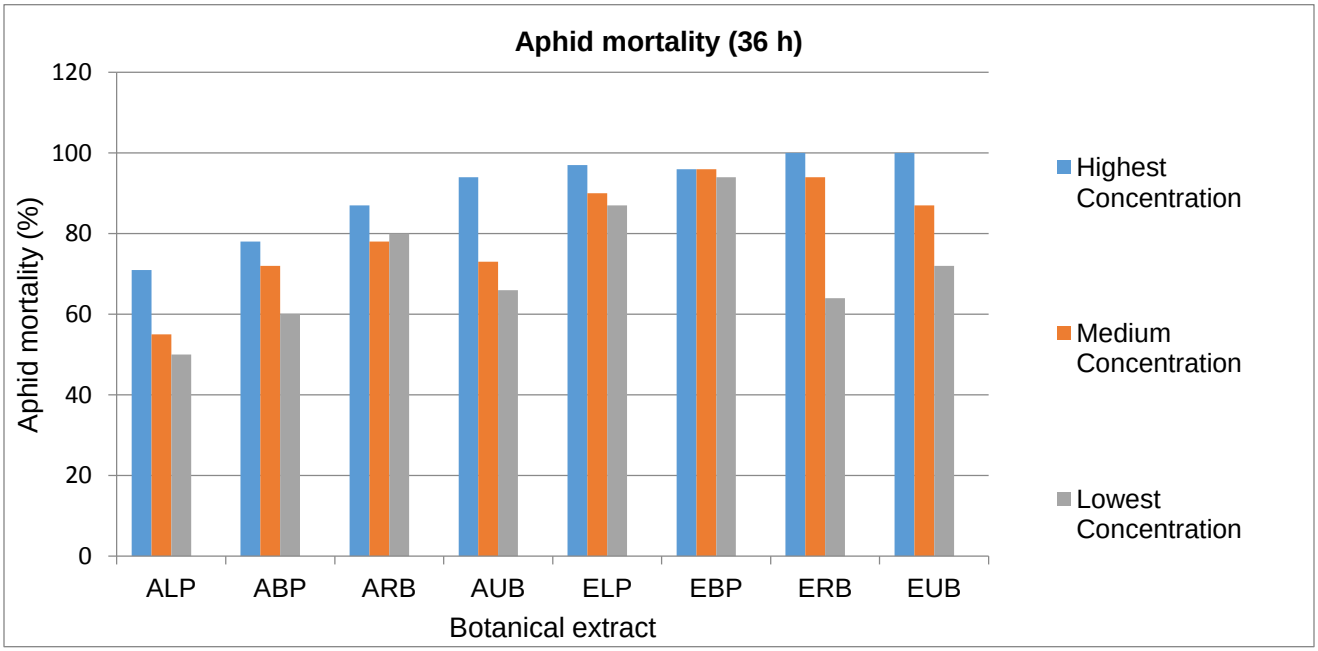

Figure 4.11: Aphid mortality after 36 h from aqueous and ethanol extracts of *S. panduriforme* at three concentration levels

ALP is aqueous leaf powder, ABP is aqueous berry powder, ARB is aqueous fresh ripe berries, AUB is aqueous fresh unripe berries, ELP is ethanol leaf powder, ERB is ethanol berry powder, and EUB is ethanol unripe berries

A factorial analysis of variance was done to determine if there was interaction between concentration level and plant part extract. The results (not shown) indicated that there was no significant interaction between extract concentration and plant parts at 12 h (P = 0.08), 24 h (P = 0.431) and 36 h (P = *0.*879). The results also showed that the extracts which were the main treatments were significantly different (P < 0.001); the concentration level was also significantly different (P < 0.001).

4.4.1.2 Discussion

A number of factors affect the effectiveness of botanical extracts; these include solvent type and concentration. In this research, mortality from the highest concentration (1:4 w/v powders and 1:40 w/v fresh berries) was highest compared to mortality from the lowest concentration (1:16 w/v berries and 1:160 w/v powders). According to Bhat & Yubak-Dhoj (2005), aqueous fresh leaf extracts of *Melea azedarach* were more effective against *B. brassicae* at 1: 5 w/v than 1:10 w/v. Mekuaninte *et al*. (2011) also found similar results; aqueous leaf extracts of *M. azedarach* were more effective against *B. brassicae* at 1% than at 0.25%. In this research aqueous unripe berries were highly effective after 36 h and significantly similar to the ethanol extracts, though all other aqueous extracts were technically effective with above 70% aphid mortality. Mekuaninte *et al*. (2011), also reported that the most effective results were obtained in the green fruit extracts with a higher concentration (74% mortality) compared to low concentration (60% mortality). In their research, the ripe fruits with lowest concentration only gave 17% mortality. The preliminary bioassays results indicated that the fresh berries extract were generally more effective after 36 h than the powdered extracts and that the immature berries were more effective than the mature berries. In this research the aqueous green fruit extracts (fresh unripe berries) also gave the highest mortality in agreement with Mekuaninte *et al.* (2011) results. The results also indicated that there was no significant interaction between the plant part and concentration. It has also been reported that a higher concentration of the product can cause a higher mortality independent of the stage of maturity or plant part (Chiffele *et al*. 2009). The results from Chiffele *et al.* (2009) and Mekuaninte *et a*l. (2011) show that effectiveness of aqueous extracts is dose dependent and this was the similar trend obtained from this research. Ntonifor *et al.* (2010) also reported that anti-feedance activity of diamond back moth larvae is dose dependant and generally increased with concentration. Aqueous extracts of tobacco, garlic and neem have been effective against tea aphids (Bahar *et al.* 2007), and aqueous extracts of *Impomea fistulata, Annona squamosal, Parthenium hysterophorus* and *Balanites aegyptiaca* were

effective against sugarcane wooly aphids (Wabale & Kharde 2010). Similarly all the aqueous extracts at the highest concentration were technically effective in the preliminary bioassays after 36 h. When water the universal solvent is used as the extraction solvent, it is important for farmers to use the most effective concentration; the results from this study indicates the highest concentration of 1:4 w/v powders and 1:40 w/v fresh berries was most effective.

In this research the ethanol extracts at the highest concentration showed significantly higher aphid mortality than aqueous extracts at the highest concentration, indicating that for the same concentration levels, ethanol extracts were more effective than aqueous extracts. All the highest concentrations (both ethanol and aqueous extracts) resulted in more than 70% aphid mortality. According to Gonzalez *et al.* (2011), botanical extracts that result in above 70% aphid mortality are considered technically effective against aphids. The preliminary bioassays results were used as the benchmark to determine the concentration to use for the subsequent bioassays.

The effectiveness of the ripe berries at the highest concentration was similar for powder extractions and fresh berries extractions using either solvent (ethanol berry powder 96% and ethanol ripe berries 100%; aqueous berry powder 64% and aqueous ripe berries 64%). The aqueous extracts resulted in 64% mortality for both ripe berry powder and fresh ripe berries and the ethanol extracts resulted in 96% and 100% mortality for ripe berry powder and fresh ripe berries respectively. This could be an indication that the ripe berries can be used either dry or fresh without affecting their effectiveness. According to Azwanida (2015) both fresh and dried plant extracts have been used in medicinal plants studies, resulting in similar effectiveness but in most cases, dried sample is preferred because fresh plant extracts are fragile, perishable and tend to deteriorate faster than dried samples. Comparison between fresh and dried *Moringa oliefera* leaves showed no significant differences in total phenolics but higher flavonoids content were found in dried materials (Azwanida 2015). This can be a great advantage to farmers who may harvest the ripe berries and young leaves of *S. panduriforme* when they are abundant and store them dry for use to control the cabbage aphid during the off season.

4.4.2: Effect of aqueous and ethanol extracts of *S. panduriforme* on aphid mortality

4.4.2.1. Subsequent bioassays results

The results from the subsequent bioassays carried out after the preliminary bioassays using three bioassays techniques (leaf dip leaf residual bioassays, aphid and leaf dip bioassays and the aphid dip bioassays), choice and no choice bioassays again showed that aqueous and ethanol extracts from *S. panduriforme* are effective against the cabbage aphid *B. brassicae*. The subsequent bioassays results are indicated in Tables 4.8 - Table 4.10. The compounds of *S. panduriforme* plant parts from the solvents used (water and ethanol) were bioactive against the cabbage aphid *B. brassicae* with varying toxicities as indicated in the following results and discussion section.

4.4.2.1.1 Leaf dip or leaf residual bioassays

The results of aphid mortality from the leaf dip leaf residual bioassays for ethanol and aqueous extracts of *S. panduriforme* showed that the plant parts have bioactive compounds that can kill the aphids as effectively as malathion. This is shown in Fig. 4.12 (a b and c), with darker coloured dead aphids observed on transferred on to treated leaf squares. The aphids were clumped at one position indicating that they did not move along the leaf to feed or to settle. The aphids that were transferred to the water treated leaf square survived as shown in Fig 4.12 (d). The aphids on the water control were spread on the leaf, were lighter coloured and showed movements when slightly touched or prodded. They maintained their waxy appearance.
The mean aphid mortality from the aqueous and ethanol extracts ranged from 18.4% for water control treated leaves to 100% for ethanol leaf powder, ethanol berry powder and malathion (Table 4.8). The results showed significant mortality differences among the ten treatments ($P< 0.05$). Aqueous leaf powder with 98% mortality was more effective than the other aqueous extracts (berry powder, ripe berries and unripe berries). The ethanol extracts did not differ significantly from each other, and generally resulted in higher mortality than the aqueous extracts which had significantly lower aphid mortality. Water control had the lowest mortality rate of 18%, indicating highest survival of aphids. Malathion had a total mortality of 100%.

Aqueous leaf powder was effective in killing the aphids as it showed a significantly similar aphid mortality to all ethanol extracts and malathion. Aqueous leaf powders have been shown to contain flavonoids and phenols which result in acute toxicity on aphids (Atteyat *et al*. 2012; Chowanski *et al*. 2016). Chiffelle *et al*. (2009) also found a high fruit fly larvae mortality rate of

90% from aqueous young leaf extracts of *M. azedarach*. Aqueous and alcohol extracts from Mexican prickly poppy leaves were also found to be effective against aphids, with aqueous extracts giving 73% mortality in laboratory bioassays and 71% mortality in the field and butanol extracts giving 100% mortality in the laboratory bioassays (Gonzalez *et al.* 2011). Water is less polar than alcohol and therefore aqueous extracts generally have less bioactive compounds than alcohol extracts (Tiwari *et al*. 2013).

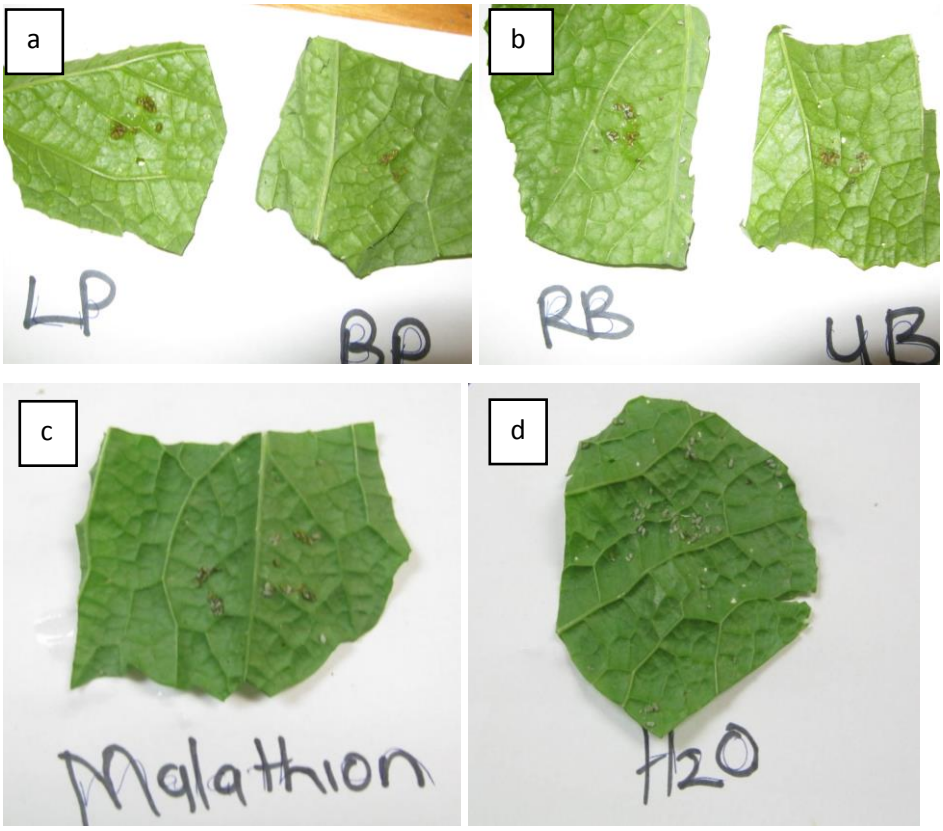

Figure 4.12: Condition and colour of aphids from leaf dip residual bioassays

a) Leaf powder (left) and berry powder (right) treatments
b) Ripe berries (left) and unripe berries (right) treatments
c) Malathion treatment
d) Water treatment

Table 4.8: Aphid mortality from leaf dip residual bioassays after 24 h

Treatment	Dilution Rate (w/v)	% Aphid Mortality
Ethanol leaf powder (ELP)	1:50 w/v	100 a
Ethanol berry powder (EBP)	1:50 w/v	100 a
Malathion 50% EC	7 ml / 5l water	100 a
Aqueous leaf powder (ALP)	1:40 w/v	97.8 a
Ethanol ripe berries (ERB)	1:4 w/v	96.7 a
Ethanol unripe berries (EUB)	1:50 w/v	96.7 a
Aqueous unripe berries (AUB)	1:4 w/v	84.1 b
Aqueous ripe berries (ARB)	1:4 w/v	81.5 b
Aqueous berry powder (ABP)	1:40 w/v	74.2 b
Water	Distilled	18.4 c

LSD at *P*< 0.05*:* 11.37 CV: 7.8%

Treatments with the same letter are not significantly different

4.4.2.1.2 Aphid and leaf dip bioassays

The 10 treatments used in the aphid and leaf dip bioassays (Table 4.9) showed that both the aqueous and ethanol extracts from *S. panduriforme* are active against *B. brassicae*.

The aphid mortality ranged from 18% for water to 100% for aqueous leaf powder, all ethanol extracts and malathion. The aphid mortality among the treatments was significantly different (*P*< 0.05). Aqueous leaf powder gave 100% aphid mortality just like malathion. The mean mortality rates from aphid and leaf dip bioassays are shown in Table 4.8.

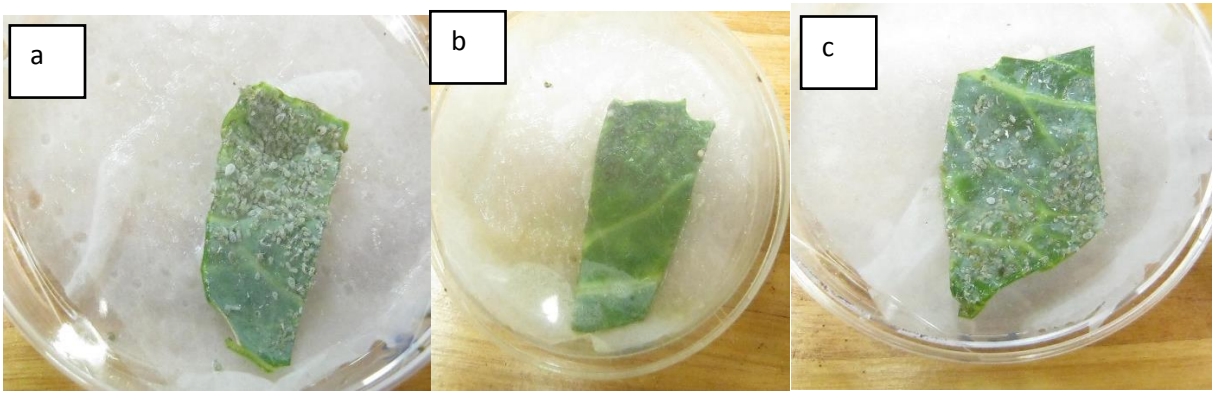

Figure 4:13: Condition of aphids from aphid and leaf dip bioassays

a) Aqueous leaf powder treatment
b) Ethanol leaf powder treatment
c) Water treatment

Table 4.9: The aphid mortality from aphid and leaf dip bioassays after 24 h

Treatment	Dilution Rate (w/v)	% Aphid Mortality
Aqueous leaf powder	1:40 w/v	100 a
Ethanol ripe berries	1:4 w/v	100 a
Ethanol unripe berries	1:4 w/v	100 a
Malathion 50% EC	7ml / 5L water	100 a
Ethanol leaf powder	1:50 w/v	100 a
Ethanol berry powder	1:50 w/v	100 a
Aqueous berry powder	1:40 w/v	91.4 ab
Aqueous ripe berries	1:4 w/v	85.1 b
Aqueous unripe berries	1:4 w/v	74.2 b
Water	Distilled	18.4 c

LSD at *P*< 0.05: 13.65 CV: 8.9%

Treatments with the same letter are not significantly different

Observations on the aphid and leaf dip bioassays on cabbage leaf cuttings showed that some parts of the cabbage leaf cuttings had not come in contact with the botanical extracts after dipping the leaf in the treatment for 5 sec as per materials and methods. This was mainly due to a combination of the waxy nature of the cabbage leaf and the cabbage aphid which results in poor surface contact. The wax may cause the insecticide to bounce off or roll of the leaf or part of the leaf (Fig. 4.13a). This is evident at the bottom of Figure 4.13a, which is showing the top part with darker colour of both the leaf and aphids and the bottom part where the insecticide could have rolled off showing lighter colour of waxy live aphids. For assessment of the effectiveness of the botanical extract this whole bottom part was discarded and the top part was considered for the assessment. Most brassica plants are waxy in nature (Dobson *et al.* 2002) and it is important for insecticides, including botanical extracts, to be prepared and applied in a way that ensures good contact as shown in Fig. 4.13c. This may be a great challenge which can result in variable effectiveness. When applying botanicals on plants a good surfactant should also be used to improve insecticide contact and they should be applied in a way that allows flowing of the insecticide to improve their effectiveness. The aphids that were dipped in the malathion control also died and the aphids from the water control (Fig. 4.13b) survived. The results from the aphid and leaf dip bioassays again showed that aphid mortality from both aqueous and ethanol leaf powder extracts were significantly similar to malathion; leaf powder was as effective as malathion in killing the aphids.

4.4.2.1.3 Aphid dip bioassays

The results from the ten treatments used for the aphid dip bioassays (Table 4.10) showed that the aphids that were dipped in the botanical extracts (both aqueous and ethanol) and malathion were all dead after 24 h with most of the aphids that were dipped in water surviving (Fig. 4.14). The aphids dipped in water were waxy, lively and healthy; they formed colonies after 24 h. Those that were dipped in the botanicals and malathion were darker and showing no movement at all (Fig. 4.14). The aphids dipped in the botanical extracts and malathion were clumped onto one position where they fell after transferring them from the tea strainer and those that were dipped in water were spread around the leaf. This shows that the botanical extracts and malathion killed the aphids as they came in contact with the insecticide.

The mean aphid mortality results from the aphid dip bioassays ranged from 3.3% for water to 100% for five out of the eight botanical extracts (Table 4.10). All the powdered botanical extracts (aqueous leaf and aqueous berry powder and ethanol leaf and ethanol berry powder) gave 100% mortality, including ethanol ripe berries. Malathion also gave 100% mortality.

The aphid dip bioassays showed that the aqueous powdered plant parts effectiveness were not significantly different from that of malathion including effectiveness from ethanol fresh unripe berries. The aqueous fresh unripe berries were significantly more effective than the fresh ripe berries which had a significantly lower mortality from all the other botanical extracts and malathion. These results showed that mature fruit extracts were less effective than the unripe immature fruit extracts and the immature leaf extracts. Mature fruits extracts have also been found to be less effective than unripe fruits extracts (Chiffelle *et al.* 2009). This may be related to the biochemical composition of immature fruitd versus mature fruits. Immature fruits have more of flavonoids with acute toxicity effects and ripe fruits have more of alkaloids with anti-feedance and deterrence effects (Chowanski *et al*. 2016). Hammami *et al.* (2011) also reported that immature fruits of *Solanum nigrum* are more effective against snails than the mature fruits; the LC 50 for mature fruits was more at 64.8 mg/L than the LC 50 for immature fruits which was 34 mg/L. In this research the same amount of *S. panduriforme* berries was used for both ripe and unripe berries unripe berries resulted in higher effectiveness for the same concentration.

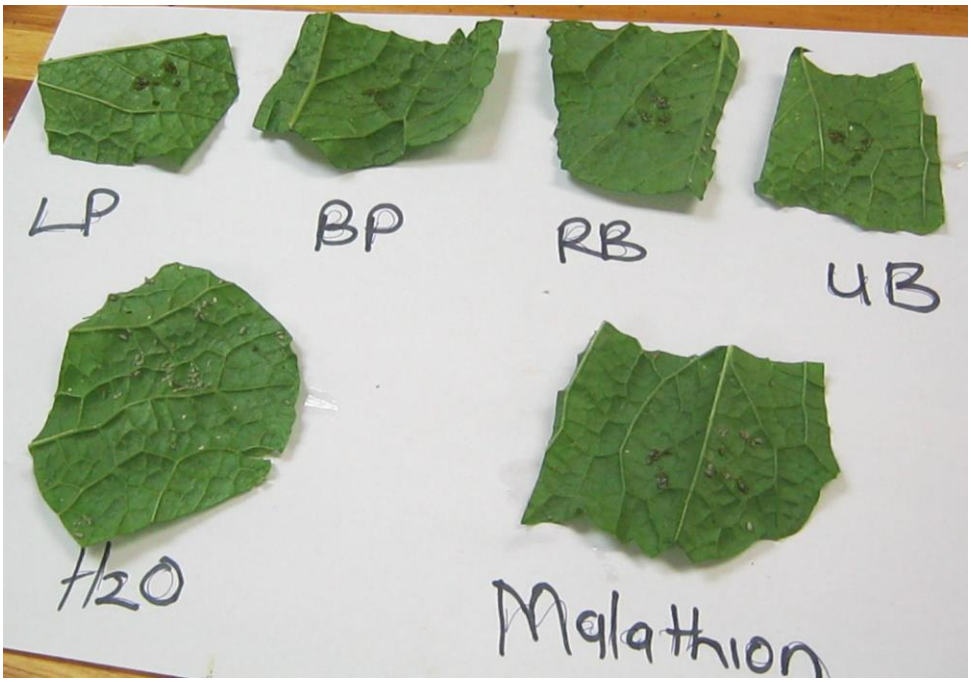

Figure 4.14: Condition and colour of aphids on aphid dip bioassays

a) Top Left to right: aqueous leaf powder, aqueous berry powder, aqueous ripe berries, aqueous unripe berries
b) Bottom Left to right: water and malathion

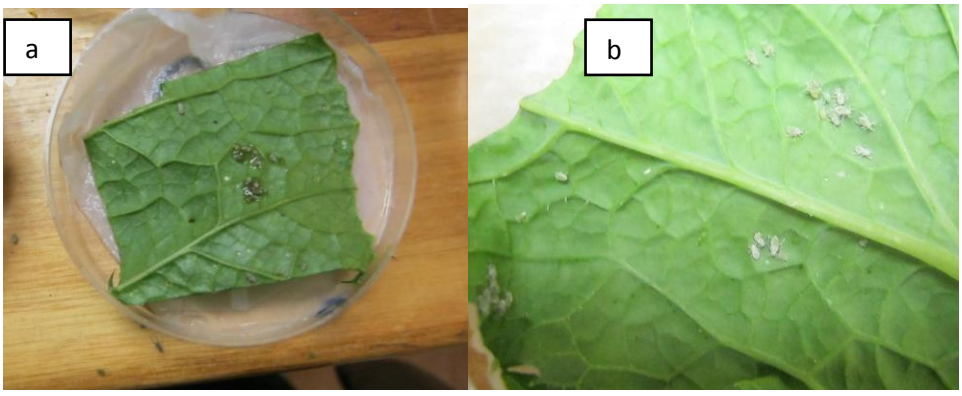

Figure 4.15: Condition of aphids on aphid dip bioassays

a) Aqueous leaf powder

b) Water

Table 4.10: The aphid mortality from aphid dip bioassays after 24 h

Treatment	Dilution Rate (w/v)	% Aphid Mortality
Aqueous leaf powder (ALP)	1:40 w/v	100 a
Aqueous berry powder (ABP)	1:40 w/v	100 a
Ethanol leaf powder (ELP)	1:50 w/v	100 a
Malathion 50% EC	7ml /5L water	100 a
Ethanol ripe berries (ERB)	1:4 w/v	100 a
Ethanol berry powder (EBP)	1:50 w/v	100 a
Ethanol unripe berries (EUB)	1:4 w/v	97.6 a
Aqueous unripe berries (AUB)	1:4 w/v	90.6 b
Aqueous ripe berries (ARB)	1:4 w/v	76.2 c
Water	Distilled	3.3 d

LSD at $P < 0.05$: 6.1 CV: 4.1%

Treatments with same letter are not significantly different

The results from the eight botanical extracts and the two controls using three different bioassay techniques showed that aqueous extracts and ethanol extracts of *S. panduriforme* have varied insecticidal properties. The ethanol extracts generally gave higher aphids mortality than the aqueous extracts (Fig. 4.16 and Fig 4.17). The aqueous leaf powder extract was similar to the ethanol extracts in the three bioassays (98% for leaf dip leaf residual, 100% for aphid and leaf dip and 100% for aphid dip) giving the highest mean mortality (Fig. 4.16). The mean aphid mortality from the three bioassay techniques showed that the aqueous powders (aqueous leaf powder and aqueous berry powder) extracts were generally more effective than the fresh berries extracts (aqueous ripe berries and aqueous unripe berries) (Fig 4.17). Aqueous leaf powder was also equally effective to malathion.

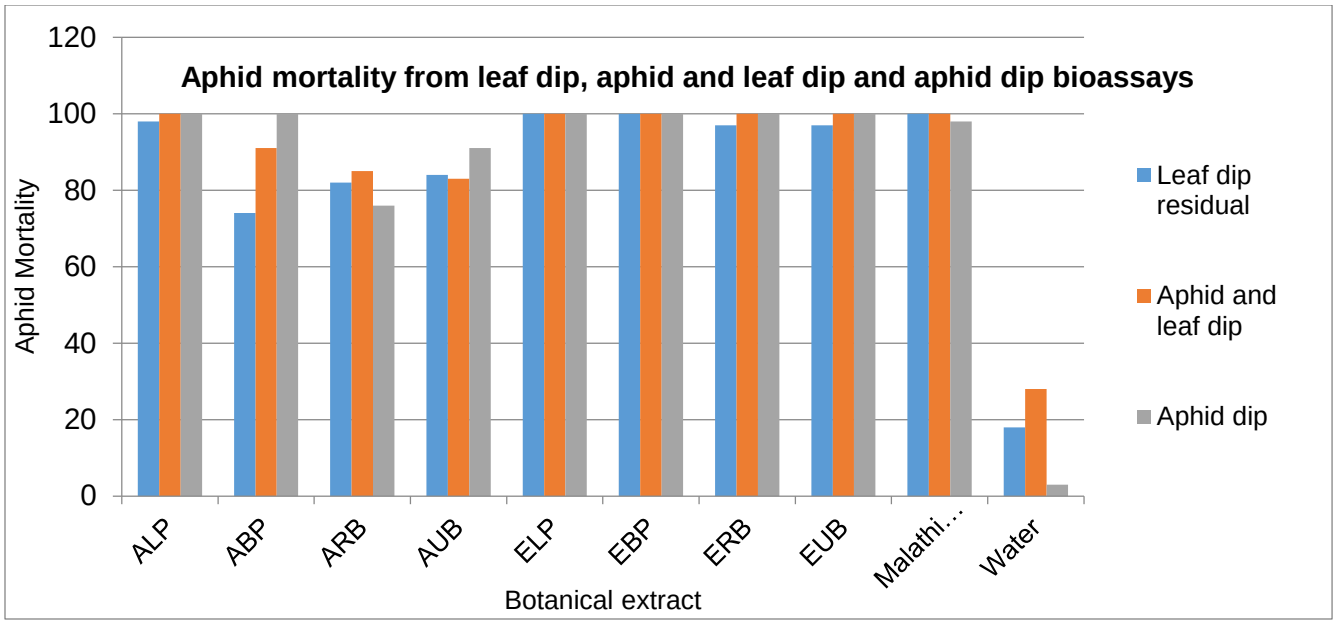

Figure 4.16: Aphids mortality from the three bioassays

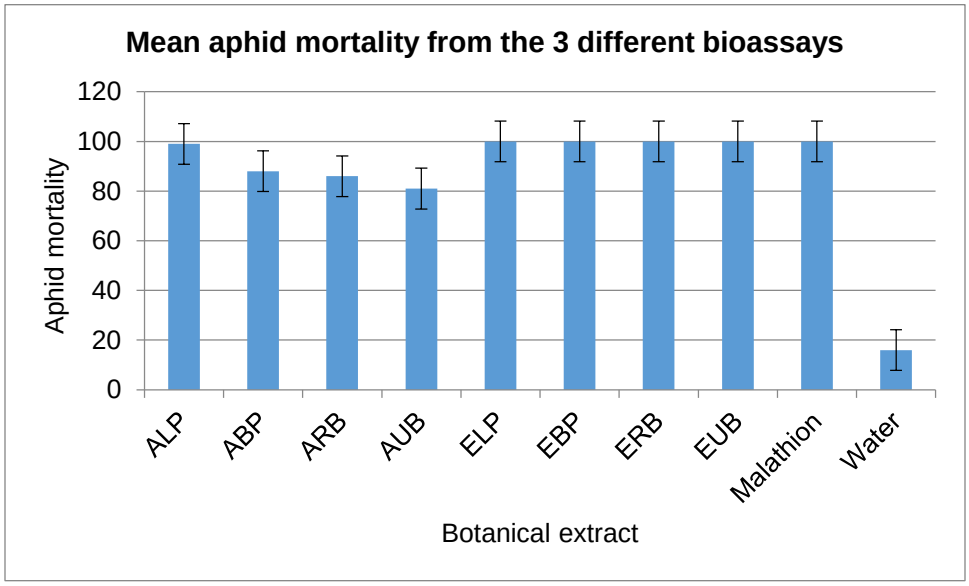

Figure 4.17 Mean aphid mortality rates for each extract from three bioassays

ALP is aqueous leaf powder, ABP is aqueous berry powder, ARB is aqueous fresh ripe berries and AUB is aqueous fresh unripe berries

ELP is ethanol leaf powder, EBP is ethanol berry powder, EBP is ethanol fresh ripe berries and EUB is ethanol fresh unripe berries

4.4.2.2 Discussion

The results from the three bioassays indicated that the aqueous and ethanol extracts from *S. panduriforme* contain bioactive compounds that are effective against the cabbage aphid. The ethanol extracts were more effective than the aqueous extracts, indicating effect of solvent. All the ethanol extracts were more effective than the aqueous extracts, and similar to malathion, except the aqueous leaf powder which was also similar to the ethanol extracts and malathion. Toxicity of ethanol extracts was also found to be most effective by other researchers. Bandeira *et al*. (2012) found ethanolic flower and fruit extracts of *Mutingia calabura* effective against the larvae and pupae of diamondback moth. Dadang *et al.* (2009) also reported that alcohol extracts are even more effective than synthetic chemicals; the extracts of *Piper retrafractum and Annona squamosa* at 0.1% resulted in a higher effectiveness against diamond back moth larvae than deltamethrin, a synthetic pyrethroid. However, Ntonifor *et al.* (2010) found no differences from the effects of water and ethanol extracts on the behaviour of insects; they found no significant differences in anti-feedance of diamond back moth from water and ethanol extracts of *Piper guineense* seeds.

In all the three bioassays, the effect of plant parts varied. Mortality from leaf powder (both aqueous and ethanol extracted) was higher than berry powder and fresh berries and similar to that of malathion. Leaf powder extracts resulted in acute toxicity, as indicated by the highest aphid mortality from the aqueous extracts, which was also similar to the ethanol berries extracts. The mean mortality from fresh unripe berries for the three bioassay techniques was lower than the mean mortality from fresh ripe berries, though the unripe berries were more effective than the ripe berries in the aphid dip and aphid and leaf dip bioassays. It has been reported that the variation in performance of botanical extracts from same plant even when prepared using the same techniques poses the greatest challenge in adoption of botanical insecticides (Isman 2008). Chiffelle *et al*. (2009) found a higher fruit fly larval mortality from young leaf extracts of *M. azedarach* than mature fruits and green fruit extracts mortality higher than mature fruits mortality. Their research also showed that young leaf extracts resulted in higher fruit fly mortality (90%) than mature leaf extracts (60%). A higher mortality of the cabbage aphid was obtained from green fruits extract than from mature ripe fruits of *M. azedarach* (Mekuaninte *et al.* 2011). Methanol extracts from mature fruits of *Solanum nigrum* were reportedly ineffective against snails than the immature fruits (Hammami *et al.* 2011). However, Bahar *et al.* (2007) reported that young mahogany fruits are ineffective against the bean aphid mortality.

This variation in effectiveness of the different plant parts is related to the nature of bioactive compounds (flavonoids, alkaloids, polyphenols and saponin) that are found in young leaves, unripe fruits and mature ripe fruits and which also depends on plant type (Benny *et al.* 2015; Hammami *et al.* 2011; Xie *et al*. 2016). Flavonoids are reported to be higher in unripe than ripe fruits; they have acute toxicity effects on aphids (Atteyat *et al.* 2012; Chowanski *et al*. 2016; Hammami *et al.* 2011). Saponins were found to be higher in mature fruits (Hammami *et al.* 2011); they have been reported to be ineffective against the cabbage aphid mortality (Habimana & Hakizayezu 2014) though Golawaska *et al.* (2008) reported that higher levels of saponin result in non-preference by the pea aphid indicating effectiveness as a deterrent. The unripe fruits and immature leaves of *S. pandurifrome* resulted in higher toxicity due to more flavonoids and the ripe berries gave lower mortality due to deterrence and antifeedance effects of saponin which also result in poor insecticide contact. In another research, aqueous and alcohol extracts from Mexican prickly poppy leaves were also effective against the green peach aphids (Gonzalez *et al.* 2011), indicating effectiveness of the leaves. Xie *et al*. (2016) reported that some fully ripe fruits have more bioactive compounds (total polyphenols and flavonoids) related to higher health care and medicinal values. Nicholson (2008) reported that highest concentration of alkaloids is found in the seed of *Solanum aculeatissimum* but both the leaves and fruits have cabbage aphid repellent effects. Some bioactive compounds are directly proportional to the maturity of the plant while others are inversely proportional (Xie *et al*. 2016).

In this research, the mortality from aqueous fresh ripe and unripe berries was generally lower than malathion but the mortality from ethanol fresh ripe and fresh unripe berries was similar to that of malathion. This could be an indication that the berries bioactive compounds are more polar in ethanol than water hence they become more effective when ethanol extracted than aqueous extracted. Leaf powder was equally effective when both ethanol and aqueous extracted, possibly indicating that the metabolites from leaves are bioactive in both solvents.

4.4.3 The effect of aqueous extracts on aphid behaviour and mortality

4.4.3.1 Choice and no choice bioassays results

4.4.3.1.1 Choice bioassays on untreated rearing leaf cuttings

Observations carried out one hour after dipping the aphid and leaf dips in the botanical extracts showed that the aphids moved from the treated aphid and leaf dip cuttings to the untreated rearing leaf cuttings (Fig. 4.18), indicating sedentary settling disturbance on aphids and preference for the untreated rearing leaf. Aphid colonies are sedentary, the aphids move only when conditions become abnormal for them (Mau & Kessing 1991; Nayar *et al.* 1990).

Figure 4.18: Aphids on choice bioassays moved from treated leaf and aphid dip (Aqueous berry powder) to untreated rearing leaf

The mortality rates for the choice bioassays are shown in Table 4.11 and Fig. 4.19. The aphid mortalities after 24 h ranged from 3% for water control to 100% for malathion. After 48 h, mortalities ranged from 8% for water to 100% for malathion and after 72 h mortalities ranged from 56% for water to 100% for malathion and berry powder. There were significant differences in aphid mortality after 12 h and after 24 h but the mortality from all the botanical extracts were not significantly different at 72 h. Aphid mortality was generally higher including that of water most likely due to rearing leaf cuttings drying out rather than from effects of the botanical extractions. The effects of the botanical extracts are mainly seen after 24 h and 48 h with the immature plant parts (leaf powder and unripe berries) being more effective and similar to malathion than the mature parts (fresh berries and berry powder). At 72 h mortality was similar for all treatments except for water which was also very high (Fig. 4.19)

Table 4.11: Aphid mortality (%) post exposure for choice bioassays (24 h, 48 h and 72 h)

Treatment	24 h	48 h	72 h
Malathion	100 a	100 a	100 a
Aqueous leaf powder	88 ab	76 ab	93 a
Aqueous unripe berries	76 abc	88 a	98 a
Aqueous ripe berries	58 bc	64 b	99 a
Aqueous berry powder	52 c	61 b	100 a
Distilled Water	3 d	8 c	56 b
LSD at $P < 0.05$	34.7 *	32.7 *	25.9 *
CV%	30.3	27.2	15.7

Treatments with same letter are not significantly different

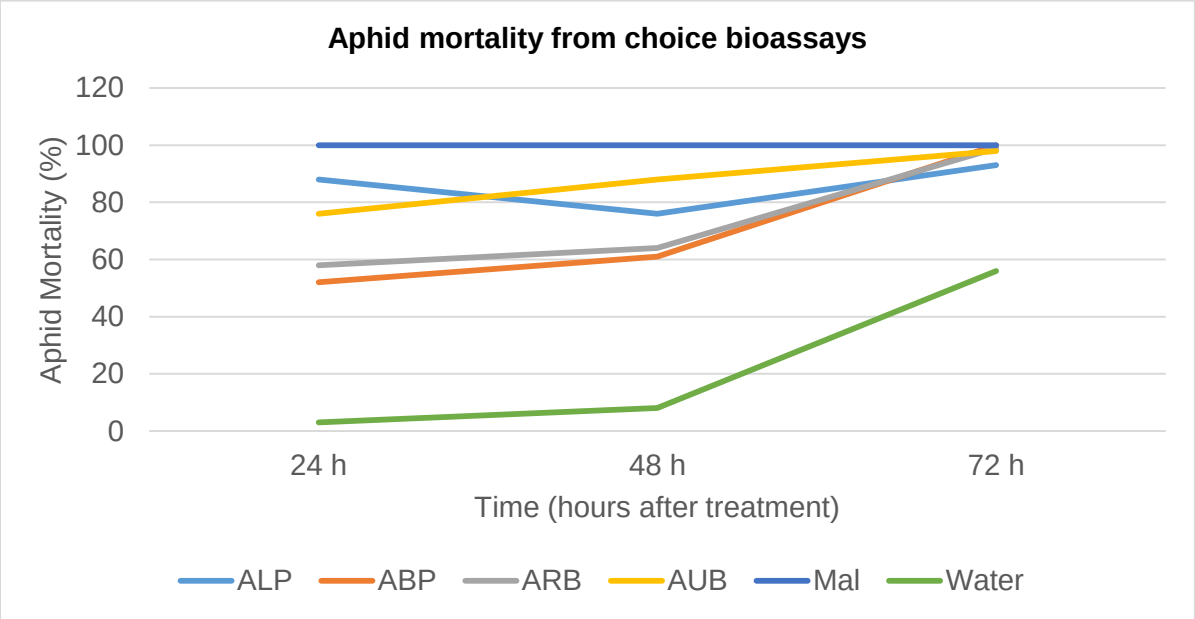

Figure 4.19 Aphid mortality from choice bioassays after 24 h, 48 h and 72 h

ALP is aqueous leaf powder, ABP is aqueous berry powder, ARB is aqueous fresh berries, AUB is aqueous fresh unripe berries and Mal is malathion

Aphid behaviour was determined by the number of aphids that moved (aphid movement) after 12 h from the treated leaf dip with aphids to the untreated rearing leaf. The percentage of aphid that moved (number aphids that moved from the treated leaf and aphid dip to the untreated rearing leaves) after 12 h ranged from 0% for water and malathion to 29% for berry powder (Table 4.12). Aphid movement was calculated as follows (number of aphids on rearing leaf divided by initial number of aphids on treated leaf dip x 100). There were significant differences on the number of aphids that moved among the botanical extracts ($P < 0.05$). Most aphid movement was from berry powder extract (29%) and the least movement was from fresh unripe berries (12%). Results from preliminary bioassays indicated that the mature fresh berries were less effective in killing the aphids; the choice bioassays also indicate the ineffectiveness of the fresh berries to kill the aphids with the effect on aphid behaviour disturbance (sedentary nature) was affected. More aphids moved from treated leaves to untreated leaves indicating a non-preference or deterrence effects. Most aphids on the treated leaf dip did not die but managed to move to a better food source. According to Akhtar *et al*. (2008) some botanical insecticides result in deterrence and antifeedance effects. The low number of aphids that moved from the immature unripe berries to the untreated rearing leaf indicate the acute toxicity effects of immature plant parts which resulted in quickly killing the aphids. There was no movement from the controls water and malathion as malathion killed the aphids on the leaf and aphid dip and therefore there was no movement. Water had no effect at all causing the aphids to settle on the treated leaves. However there were more aphids moving from the powdered extracts than the fresh berries (Fig 4.20). This could be an indication that the powders have a knock down effect which may result in more disturbed settling of the aphids before they die or survive.

Table 4.12: Aphids moving from treated aphid and leaf dip to untreated rearing leaf

Treatment	**% Aphids moving to untreated rearing leaf** (Numbers in brackets are transformed figures)
Aqueous berry powder	29 (1. 441) a
Aqueous leaf powder	19 (1.204) ab
Aqueous ripe berries	18 (1.170) ab
Aqueous unripe berries	12 (0.826) b
Malathion	0 (0) c
Water	0 (0) c

LSD at $P < 0.05$: 1.8 (0.591) CV: 15.7 %

Treatments with the same letter are not significantly different

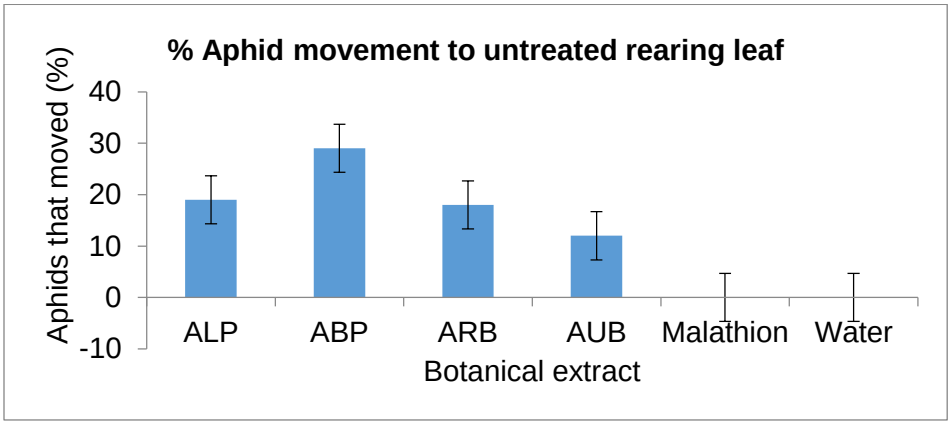

Figure 4.20: Aphids that moved from treated aphid and leaf dip to untreated rearing leaf

ALP is aqueous leaf powder, ABP is aqueous berry powder, ARB is aqueous fresh ripe berries and AUB is aqueous fresh unripe berries

Some of the effects of Solanaceae plant compounds which include non-preference, acute toxicity and disturbed settling or effects on behaviour, according to Chowanski *et al. (*2016) are reflected in the choice bioassays. The aphids had a choice of treated and non-treated leaves; those that survived on less toxic extracts (aqueous berry powder and aqueous ripe berries) moved to the untreated rearing leaf. Under normal situations aphids are sedentary during their life time (Kessing & Mau 1991; Mau & Kessing 1991). Mature berries are known to have low glycoalkaloid levels (Gonzalez 1997), with insect repellant properties, thus the movement from the mature berries was probably due to their repellency effects. The leaf extracts of *Lycium cestroides* were also reported to inhibit settling of the green peach aphid (Chowanski *et al.*

2016). The lowest number of aphids moved from aqueous unripe berries and aqueous leaf powder which also had the highest mortality indicating the high acute toxicity of the immature plant parts. The effects of *S. panduriforme* on the cabbage aphid thus include non-preference, acute toxicity and disturbed settling as indicated by mortality and aphids moving away from treated to untreated leaves. Chiffelle *et al.* (2009) and Mekuaninte *et al*. (2011) also reported that immature fruits and leaves are more toxic than mature fruits and mature leaves.

4.4.3.1.2 No-choice bioassays on treated rearing leaf cuttings

In the no choice bioassays using the aphid and leaf dip bioassays, the rearing leaves were also dipped in the same treatment. Observations of aphids' movement from the treated aphid and leaf dip leaves to the treated rearing leaves showed that after 12 h, most aphids had not moved from the treated aphid and leaf dip cutting to the treated rearing leaf cuttings as was observed in the choice bioassays where aphids were reared on untreated leaves. The aphids from aqueous leaf powder were clumped on the leaf and aphid dip showing no movements at all (Fig 4.21a).They had slight dull colour changes, the waxy nature disappeared and those that had moved were on the moist cotton wool (Fig. 4.21b). The aphids from ripe berries were not much clumped but slightly dispersed on the treated rearing leaf and aphid dip (Fig 4.21c) and aphids from unripe berries were slightly dispersed with most aphids dead on the aphid and leaf dip (Fig. 4.21d). Aphids from malathion were slightly dispersed on the rearing leaf, most were dead on the aphid and leaf dip soon after dipping (Fig.4.21e). Aphids from water treatment showed a normal waxy colour (Fig. 4. 21f).

Some of the aphids moved from the aphid and leaf dips to the lids of the Petri dishes; aphids could be seen on the Petri dish lids and on the moist kitchen paper towel during assessments at 24 h and 48 h. During mortality assessment checks, the aphids from the fresh ripe berries and water treatment remained stationary after being prodded, further observation using a magnifying glass revealed that their legs showed some movements. This probably indicated that they were feeding because their stylets seemed stuck into the leaf. After 24 h the surviving aphids on treated leaf cuttings from the leaf powder, unripe berries and ripe berries showed weaker movements when prodded than those from the water treatment. Observations of aphids from the ripe berries (both fresh and powder) showed that some aphids had developed wings, indicating need to migrate to start new colonies on other favourable hosts. Some adults from water treatment and fresh ripe berries had produced nymphs after 48 h (Fig 4.21f).

The mortality results from the no-choice bioassays which were repeated as bioassay 1 bioassay 2 and bioassay 3 are shown in Table 4.13. The aphid mortality after 24 h ranged from 9% for water in bioassays 1 to 100% for malathion and after 48 hours mortality ranged from 22 % for water in bioassay 3 to 100 % for malathion. There were significant differences on the aphid mortality ($P < 0.05$). The mean aphid mortalities for all the no-choice bioassays are shown in Fig. 4.24. The results indicate that immature parts (aqueous leaf powder and aqueous unripe berries) are more effective than the mature parts (aqueous berry powder and aqueous ripe berries). Aqueous leaf powder (90%) and aqueous unripe berries (83%) were more toxic than aqueous ripe berries (74%) and aqueous berry powder (67%).

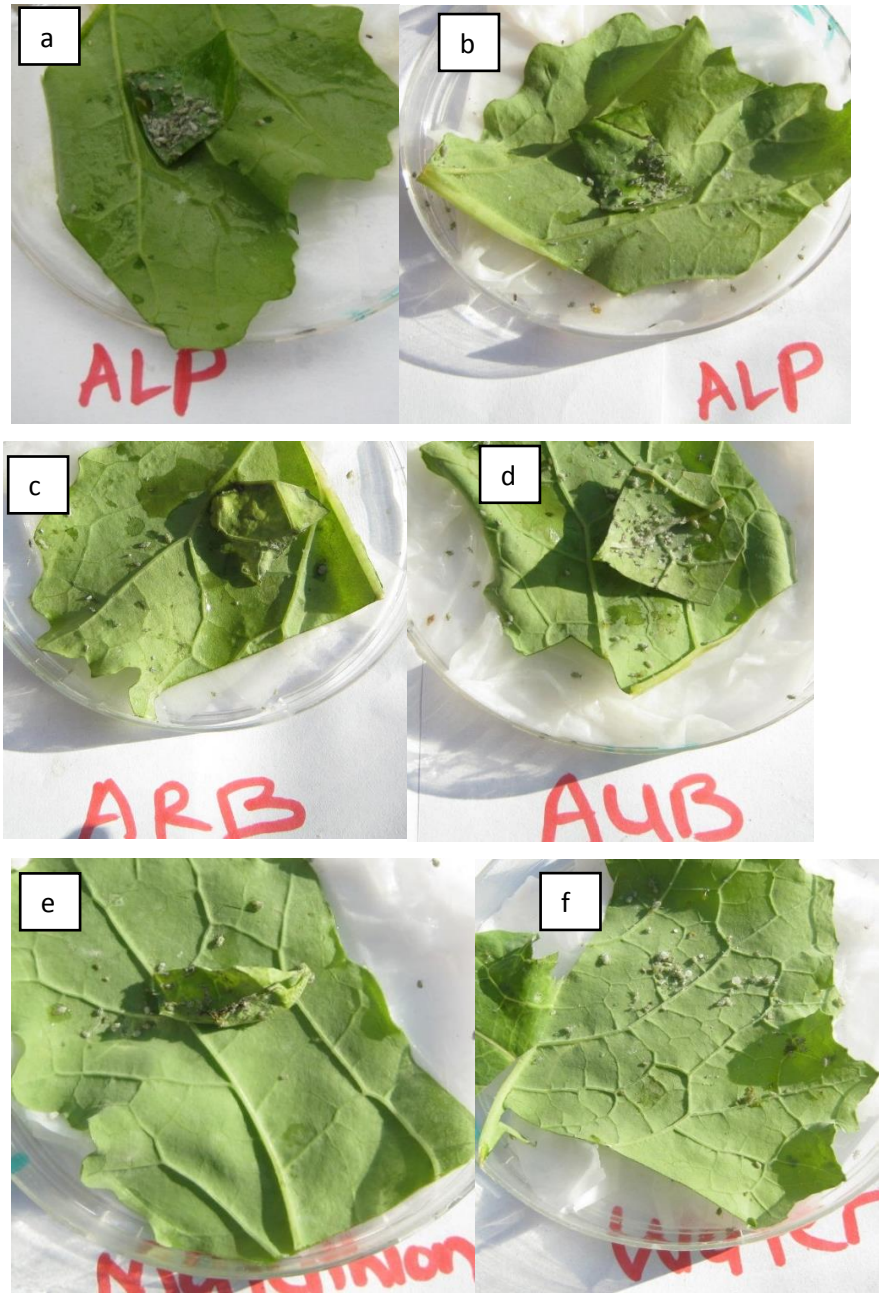

Figure 4.21 No choice bioassays

a) No aphid movement from treated leaf and aphid dip (aqueous leaf powder)
b) Aphid movment to moist kitchen towel (aqueous leaf powder)
c) Aphid movement from treated leaf dip to treated rearing leaf (aqueous ripe berries)
d) Slight to almost no aphid movement from treated rearing aphid and leaf dip to treated rearing leaf and moist kitchen paper (aqueous unripe berries)
e) No movement and dead aphids (malathion)
f) Aphid movement from water with nymphs produced at bottm right after 36 h (water)

Table 4.13: Aphids mortality rates for no choice bioassays

Treatment	Bioassay 1 Mortality (%)		Bioassay 2 Mortality (%)		Bioassay 3 Mortality (%)		Mean Mortality (%)	Mean Mortality (%)
	24 h	48 h	24 h	48 h	24 h	48 h	24 h	48 h
Leaf powder	87 b	93 ab	74 ab	92 ab	93 a	100 a	84	95
Berry powder	77 c	73 c	47 bc	61 abc	46 c	71 b	57	68
Ripe berries	80 bc	78 bc	51 b	57 bc	71 b	98 a	67	78
Unripe berries	97 a	91 ab	49 bc	55 bc	83 ab	97 a	76	81
Malathion	100 a	96 a	100 a	100 a	93 ab	95 a	98	97
Water	9 d	22 d	19 c	31 c	20 d	40 c	17	31
LSD at $P < 0.05$	9.8*	16.4*	31.1*	42.2*	21.6*	11.6*		
CV (%)	7.16	11. 88	30.16	35.25	17.57	7		

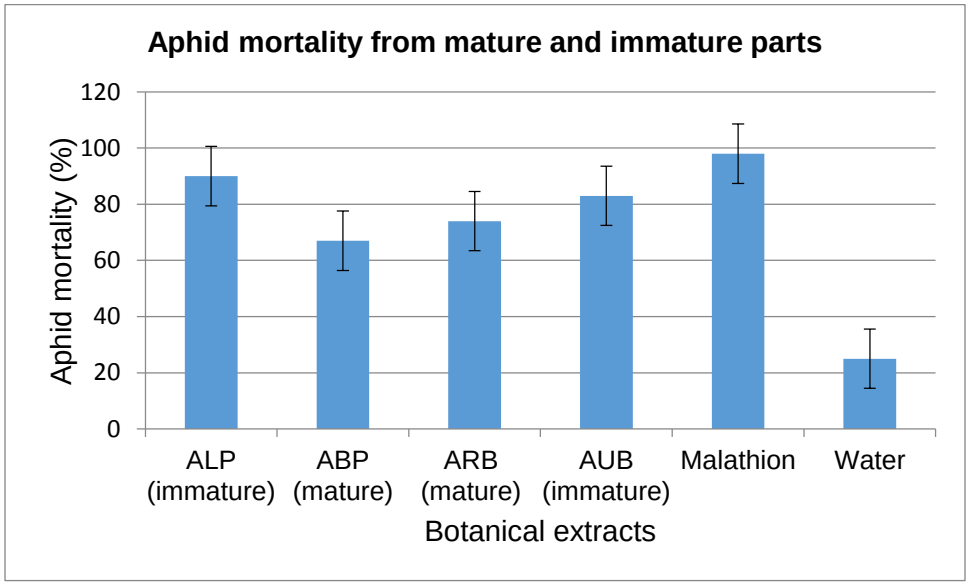

Figure 4.24: Mortality effects of aqueous extracts from mature and immature parts of *S. panduriforme* on the cabbage aphid mortality in no choice bioassays

ALP is aqueous leaf powder, ABP is aqueous berry powder, ARB is aqueous fresh ripe berries, and AUB is aqueous fresh unripe berries.

4.4.3.2 Discussion

The results from the choice and no choice bioassays are similar with the subsequent bioassays (Fig 4.17) and in agreement with other researchers. The immature plant parts (aqueous leaf powder) and unripe berries (aqueous unripe berries) had higher aphid mortality and thus were more effective than the mature plant part (aqueous berry powder and aqueous ripe berries). Chiffele *et al.* (2009) found a higher mortality of *Drosophilla melanogaster* from aqueous green fruits extracts than mature ripe fruits of *Melea azedarach*. They also found that both the green fruits and young leaf extracts were more effective, reaching mortality rates of 90%. Ripe fruits of *Solanum americanum* and *Solanum carolinense* were preferred as a food source by seed dispersers and predators as compared to unripe fruits (Cipollini & Levey 1997). The ripe fruits contain much lower levels of the two main glycoalkaloids α-solasonine and α-solamargine found in *Solanum* plants (Matu 2008; Nicholson 2008). Many alkaloids act as potential insect feeding deterrents and are generally not favoured by insects (Chowanski *et al*. 2016). Extracts of leaves and fruits of *Solanum aculeatissimum* were reported to have repellent effects on the cabbage aphid (*Brevicoryne brassicae*) (Nicholson 2008).

However, Aziz *et al.* (2013) carried out laboratory bioassays and found neem leaf extract (NLE) and neem cake extract (NSCE) to be least effective against English grain aphid when compared to neem seed kernel extract (NSKE) under field conditions. This is in contrast with the results of this study, where leaf powder extract was more effective than ripe berry powder which included the seeds as well. The variation may be due to differences in biochemical components of neem leaves versus *S. panduriforme* leaves. As mentioned earlier, the effectiveness of botanical extracts depends on a number of factors which include plant type, solvent type and conditions of application (Tiwari *et al.* 2011).

4.4.4: Effect of solvent assisted (hexane, diethyl ether and water) extracts on aphid mortality

4.4.4.1 Topical bioassays results

4.4.4.1.1 The solvent assisted /sequential extraction dried extract amounts

The dried crude extracts amounts successively extracted from 10 g powder in 400 ml solvent and 100 g fresh berries in 400 ml solvent (hexane and diethyl ether) are shown in Table 4.13 as solvent based and plant part based. The highest amount of crude extracts was from unripe berries diethyl ether extract with 5.4 mg and the lowest amount was from unripe berries hexane extract with 0.8 mg. The unripe berries had highest combined extract yield with 6.222 mg and the lowest combined extract yield was from leaf powder with 2.409 mg.

Table 4.13: Extract amounts from solvent assisted extraction per solvent and plant part

Plant part	**Extract amount (mg)**		
	Hexane	**Diethyl ether**	**Total per plant part**
Leaf powder	1.2749	1.1348	2.409
Berry powder	1.356	3.8308	5.1847
Fresh ripe berries	2.1041	2.8684	4.972
Fresh unripe berries	0,805	5.4146	6.222

4. 4.4.1.2 Aphid mortality from topical bioassays

The topical bioassays carried out using dried crude extracts dissolved in acetone indicated that hexane and diethyl ether dried crude extracts from leaf powder, berry powder, fresh ripe berries and fresh unripe berries were highly toxic to the aphids (Fig. 4.25). The aphid mortality ranged from 37% for water contol and acetone to 100% for all hexane extracts and diethyl ether extracts of the immature plant parts (leaf powder and unripe berries) as shown in Table 4.14. There were significantly differences ($P < 0.05$) in aphid mortality from the effects of the solvents and the plant parts as well. The aqueous extracts generally gave lower aphid mortality than the hexane and diethyl ether extracts, which gave almost 100% mortality. However aqueous unripe berries gave a significantly higher aphid mortality of 94%, indicating effectiveness of the unripe berries from all the solvents (hexane; 100%, diethyl ether; 100% and water 94%). The immature

unripe berries were highly toxic and this was in agreement with the subsequent bioassays. The hexane extracts were highest followed by diethyl ether extracts, with aqueous extracts showing the least effectiveness (Fig 4.26). The powdered aqueous extracts gave lower mortalities than the fresh berries, the results are similar with the preliminary bioassays but different from the subsequent bioassays in which the powdered extracts gave higher cabbage mortalities. This problem of variable effects of botanicals has been reported as one of the drawbacks of use of botanical insecticides by farmers (Isman 2006; Isman 2008). The topical bioassays results indicate the aphicidal effects of leaf powder, berry powder and fresh ripe and unripe berries of *S. panduriforme* from solvent assisted extraction in terms of acute toxicity.

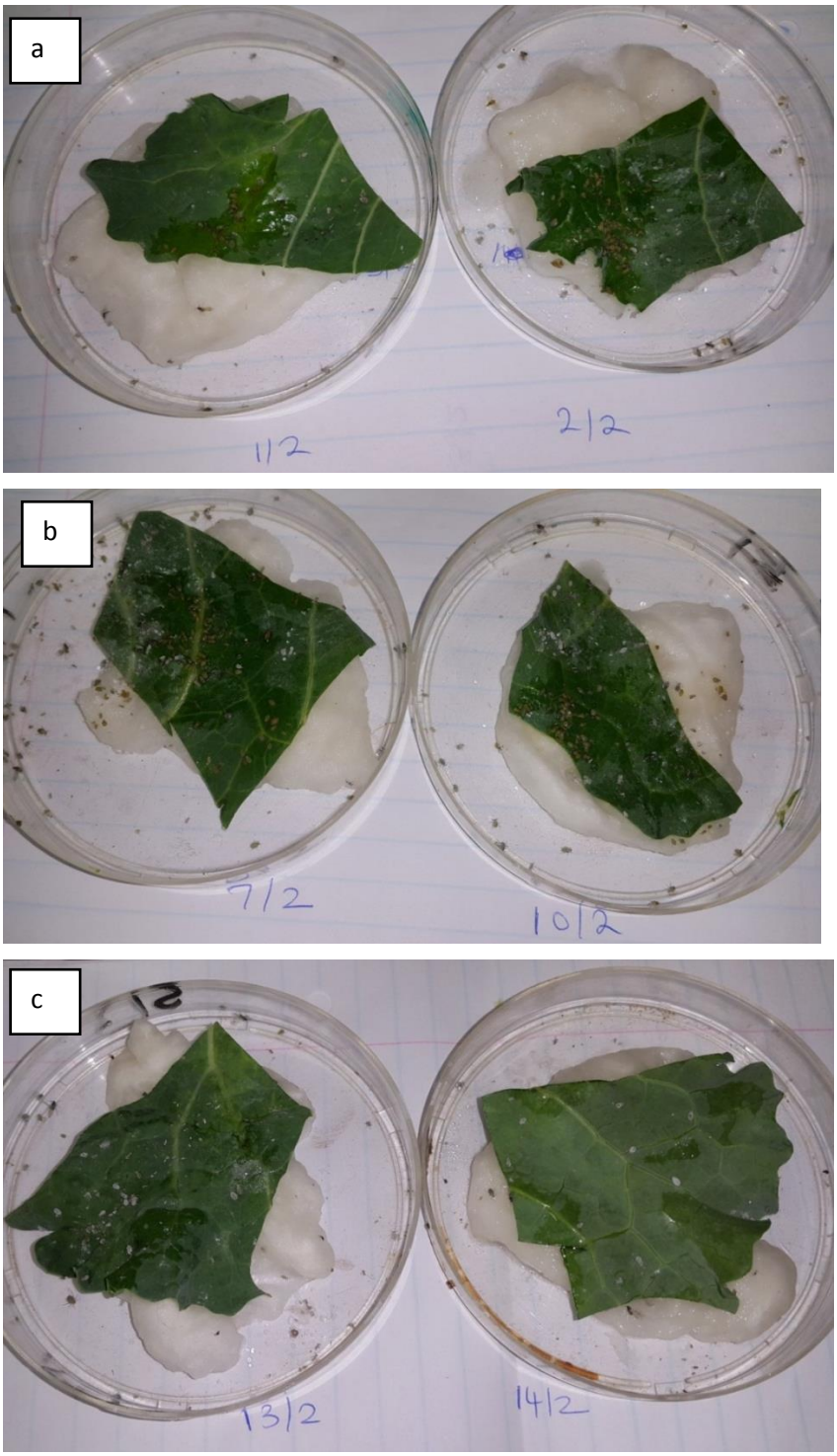

Figure 4.25: Topical bioassays

a) Aphids on leaf powder extracts hexane (right) and diethyl ether (left)
b) Aphids on hexane extracts ripe fresh berries (left) and fresh unripe berries (right)
c) Aphids on controls acetone (left) and water (right)

Table 4.14: Aphid mortality from hexane and diethyl ether dried crude extracts

Extract	**Aphid Mortality (%)**
Leaf powder hexane (LPH)	100 a
Leaf powder diethyl ether (LPEE)	100 a
Berry powder hexane (BPH)	100 a
Fresh berries hexane (RBH)	100 a
Fresh unripe berries hexane (UBH)	100 a
Fresh unripe berries diethyl ether (UBEE)	100 a
Berry powder diethyl ether (BPEE)	98 a
Fresh unripe berries water (AUB)	94 ab
Fresh berries diethyl ether (RBEE)	83 bc
Fresh berries water (ARB)	78 cd
Berry powder water (ABP)	74 cd
Leaf powder water (ALP)	68 d
Acetone	37 e
Water	37 e

LSD at $P < 0.05$: 13 CV: 9.6%

Treatments with the same letter are not significantly different

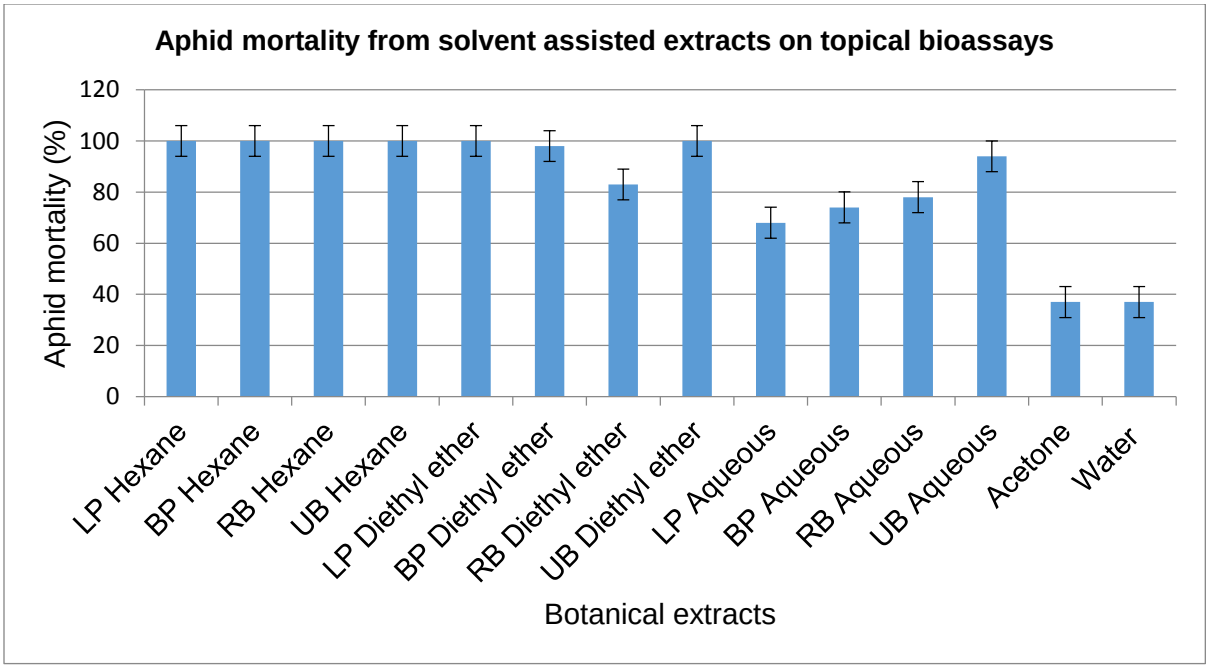

Figure 4.26 Aphids mortality showing effects of solvent and plant part from solvent assisted extraction method

LP is leaf powder, BP is berry powder, RB is fresh ripe berries and UB is fresh unripe berries.

4.4.4.2 Discussion

The results from the topical bioassays were in agreement with the preliminary (Fig 4.11) and subsequent (Fig. 4.17) bioassays. The results showed that immature plant parts are generally more effective than mature plant parts. The aphids from the dried crude extracts died within an hour after being transferred on to the Petri dishes for the topical bioassays; an indication that the dried crude extracts from hexane and diethyl ether were highly toxic. Depending on their polarity, different solvents extract varying quantities of components in crude plant material that may have beneficial or harmful effects to biological systems (Tiwari *et al.* 2011; Sasidharan *et al.* 2011). In this research, the hexane crude extracts were most effective with highest aphid mortality and the aqueous extracts least effective with lowest aphid mortality. This is because water soluble extracts have less significant bioactive activities when compared to organic solvents (Tiwari *et al*. 2011). Other researchers also found hexane crude extracts to be most effective (Fan *et al.* 2011; Soliman *et al.* 2005; Tennyson *et al.* 2011). The hexane extract of *Piper nigrum* fresh fruits was most effective in killing the larvae of *Spodoptera litura,* showing the highest toxicity after 48 h; toxicity of the extracts decreased in the order of hexane > acetone > chloroform (Fan *et al.* 2011). Hexane and acetone were found to be the best solvents to extract or elute chemical compounds with acaricidal and aphicidal activities from bitter apple (*Citrullus*

colocythis) and Jews mallow seed (*Corchorus obitorius*) (Mustafa & Gazi 2009). Tennyson *et al. (*2011) found the ovicidal activity of hexane crude extracts to be highly effective than diethyl crude extracts on mosquitoes eggs. Ethanol plant extracts proved superior efficacy against *A. gossypii* followed by ethyl acetate and diethyl ether plant extracts were the least effective (Soliman *et al*. 2005). In this study the ethanol extracts of *S. panduriforme* were also more effective against the cabbage aphid *B. brassicae* mortality when they were tested in the preliminary bioassays using three concentrations and in the subsequent bioassays using three different bioassays. The diethyl ether extracts were also generally less effective when tested using topical bioassays. However, Nissar *et al*. (2012) concluded that organic solvent or aqueous extracts of leaf and seed of *Jatropha curcas* exhibit similar effects on termites in terms of toxicity and inhibition of tunneling and both can be used to control termites.

According to McLaughlin *et al*. (1998), for bioactive botanicals to be accepted and incorporated into legitimate long-term practices, there is a need to screen and identify bioactive compounds using readily available technologies which must be combined (Stell *et al*. 2013). The readily available technologies for screening bioactive compounds from plant materials include simple bioassays, separation techniques (chromatography and non-chromatography) and structural elucidation methods (spectrometry). The extracts must be analysed many times over and over again after pre-screening with general bioassays to ascertain their bio-effectiveness (Stell *et al*. 2013). In this research, it was logical to pre-screen with general bioassays on different dates; the same extracts were analysed over and over because bioassays trials need replication.

4.5 Laboratory bioassays conclusion

The repetitive bioassays to evaluate the effectiveness of extracts from *S. panduriforme* on the cabbage aphid using preliminary bioassays, subsequent bioassays, choice and no choice bioassays and topical bioassays indicated that aqueous and ethanol extracts of *S. panduriforme* from the leaves and berries are effective against the cabbage aphid but with variable effects. The preliminary bioassays to evaluate effect of concentration showed that the extraction rates of 1:4 w/v fresh berries and 1:40 w/v powders can achieve technically effective aphid mortality rates of above 70%. The subsequent bioassays carried out to evaluate the effect of extracts from two solvents (water and 60% ethanol) showed that ethanol extracts are more effective than aqueous extracts, though the immature parts (aqueous leaf powder and aqueous unripe berries) were also highly effective. The choice bioassays and no choice bioassays indicated that aqueous extracts from the mature berries (berry powder and fresh ripe berries) have settling

inhibition or disturbed aphid behaviour effects; the immature parts (leaf powder and unripe berries) have acute toxic effects. The topical bioassays indicated that both hexane and diethyl ether dried crude extracts have very high acute toxicity effects though hexane crude extracts are more effective than diethyl ether crude extracts against the cabbage aphids on brassicas. The immature plant parts (leaf powder and unripe berries) were generally more effective against the cabbage aphids than the ripe berries in terms of acute toxicity. The mature berries had more of deterrence effects than acute toxicity effects on the aphids. The extracts from the plant also showed effects of disturbed reproduction and disturbed settlement as was indicated by development of winged adults for migration and failure by the aphids to settle to establish colonies. The berries and leaves of *S. panduriforme* may be used to produce home-made aqueous botanical insecticides. Aqueous extracts from leaf powder and the unripe berries can be extracted using homogenisation and maceration respectively; the extracts may be used directly by resource poor farmers considering that bioassays results showed that these plant parts were generally more effective. There is a possibility of using the plant to extract active ingredients for production of a commercial botanical insecticide since the bioassays results indicated that the plant is effective against the cabbage aphid mortality. However, on farm research is needed to evaluate the effectiveness of the extracts under field conditions while assessing the botanical extract effects on beneficial insects.

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CHAPTER 5: EVALUATION OF APHICIDAL EFFECTS OF *SOLANUM PANDURIFORME* EXTRACTS ON *BREVICORYNE BRASSICAE* USING PLANT ASSAYS

5. 1 Introduction

The effectiveness of botanical plants on insects requires evaluation in both laboratory and field trials (EFSA 2009). Vigorous and robust field studies are essential when evaluating botanicals because they more closely resemble the situations where the products are to be used. Simulated-use tests designed to mimic the practical use situation can also be used (European Commission, 2010), depending on the insect to be tested. The simulated use trials may involve artificially infesting plants in the field or screen-house to get a clear picture of the effects from the intended use of the product, or use of test chambers with insects to introduce the botanical insecticides. The test insects may be given a choice to be in contact with the botanical insecticide or not. The simulated-use tests to be used should result in or should provide a clear picture of the effectiveness of the product (Kranthi 2005).

Direct spray applications of botanical extracts from leaves, stem, roots, fruits and whole plants are now used to control soft bodied insect pests, such as aphids, jassids and even caterpillars (Prakash *et al*. 2008; Varela *et al*. 2003). Many of the botanicals are used as insecticides both in homes, commercial and subsistence agriculture by small-scale farmers (Kareru *et al*. 2013). The botanicals may be contact, respiratory or stomach poisons (Bogran *et al*. 2011). Some commercial botanical insecticides from neem, pyrethrum, pepper and sabadilla have been recently developed and attained the status of potential pesticides of plant origin, and are used in integrated pest management (IPM) of field and storage insect pests (Dubey *et al.* 2010; Prakash *et al.* 2008; Varela *et al*. 2003). However, botanical insecticides play a minor role in insect pest management and crop protection in Africa, since farmers prefer to use synthetic insecticides (Kareru *et al*. 2013; Stevenson *et al.* 2012), even though successful control of insects with botanicals has been recorded for a limited number of plants. According to Prakash *et al*. (2008) aphids can be controlled using botanicals; losses due to mustard aphid *(Lipaphis erysimi)* have been minimized by spraying neem leaf and neem kernel extracts; spraying neem oil (1.5%) resulted in 100% mortality of the aphids. They also reported that leaf extracts of neem and *Annona squamosa (*12%*)* have strong anti-feedant effects against the cabbage aphid, *B. brassicae* and that neem oil (0.5%) sprayed on cauliflower show repellent effects to the cabbage aphid.

It has been reported by Stevenson (2010) that farmers sometimes use ripe berries of *S. panduriforme* to control insect pests on vegetables like tomatoes and some brassicas. Masingi *et al.* (2008) also reported that some farmers use the berries for veterinary purposes to control nasal worms on sheep. Laboratory-based bioassays in the current research showed that extracts from the leaves and unripe berries contain bioactive compounds. The bioassays carried out on *B. brassicae* showed that the plant extracts from *Solanum panduriforme* caused mortalities of the cabbage aphid under laboratory conditions on cabbage, mustard rape and kale plants (current study: Chapter 4). The objectives of the study were to assess the aphicidal effects of aqueous extracts of *Solanum panduriforme* from leaf powder, berry powder, fresh ripe berries and fresh unripe berries on the mortality of the cabbage aphid *Brevicoryne brassicae* on young seedlings and mature *B. napus* plants.

5.2 Materials and methods

5.2.1. The plant materials

The plant materials used were dried young leaves, dried ripe berries, fresh ripe berries and fresh unripe berries of S*olanum panduriforme* collected from an abandoned field at the University of Venda in Limpopo Province, South Africa (22.9761° S, 30.4465° E). Plant materials were processed and extracted as outlined in Chapter 3 of the current study.

5.2.2 Plants assays

The plant assays were carried out using seedlings and established *B. napus* plants (Pavela 2009; Sallam *et al*. 2009; Wabale & Kharde 2010). The plants were planted in pots (seedlings) in a net house and in the field (mature established) (Fig. 5.1 and 5.2). Rape seeds were sown directly in pots, small kaylite and bigger plastic pots were filled with potting media (organic mixture of manure, pine bark and garden soil). After germination, the plants were thinned to two plants per pot. The thinned seedlings were planted in the field to raise the field plants according to rape production agronomic recommendations (Toxopeus & Mvere 2004). The plants were watered regularly during the trial period. The completely randomised design (CRD) with six treatments was used for the plant assays (Table 5.1). Three replications were used for the pot plants and four replications for the field plants.

Table 5.1 Treatments used for the plant assays

Treatment	Extract	Concentration
1	Aqueous leaf powder (ALP)	1:40 w/v
2	Aqueous berry powder (ABP)	1:40 w/v
3	Aqueous ripe berries (ARB)	1:4 w/v
4	Aqueous unripe berries (AUB)	1:4 w/v
5	Malathion 50% EC	7 ml / 5 l water
6	Water	Distilled

The pot plants were initially infested with 15 - 20 aphids which were allowed to develop into colonies. Three plants with high aphid infestation were selected for each treatment for the pot plants assays; each plant constituted a replication. Leaf sections with aphids were marked on each selected plant to record number of aphids before spraying. The pot plant assays were repeated twice since the first spray resulted in high aphid mortality after 24 h (above 80%) for all the extracts.

The field plants were allowed to be naturally infested with aphids. Four plants with high aphid infestation were selected. Leaf sections with aphids were marked on each selected plant to record the number of aphids before spraying. Three hand sprayers were used (Fig 5.3), one was specifically for water, one for malathion and another for the botanical extracts. For the botanical extracts, the sprayer and nozzle were thoroughly rinsed after each treatment. The plants were sprayed until the spray flowed off or dripped off the leaf (drenching). The number of live aphids was recorded on each sprayed potted and field plant after 24 h and 72 h. The percentage of live aphids on plants and percentage aphid mortality were calculated.

Figure 5.1: *B. napus* seedlings in pots for plant assays in a net house

Figure 5.2: *B. napus* plants infested with aphids used for plant assays in the field.

Figure 5.3: Spraying aphid infested *B. napus* plants with the botanical extracts.

5.3. Plant assays results

The leaf and berry extracts from *S. panduriforme* sprayed on the rape plants infested with aphids resulted in death of aphids as shown in Figs. 5.4– 5.9 which are showing the infested leaves before and after spraying. There were significant differences ($P < 0.05$) in aphid mortality among the treatments. Table 5.2 shows the mean aphid mortality from pot plants for the first spraying for the four aqueous treatments and the two controls at 24 h; Table 5.3 shows the mean aphid mortality after 24 h and 72 h for the second spraying. The first spraying resulted in above 80% aphid mortality after 24 h for all the treatments except water (11%). All the botanical extracts were technically effective after 24 h. The second spraying resulted in aphid mortality of 13% for water with a low mortality of 53% from aqueous unripe berries to 100% for malathion after 24 h and 20% for water to 100% malathion after 72 h (Table 5.3). There were significant differences in aphid mortality among the botanicals and the two controls ($P < 0.05$). All the botanical extracts were effective against the cabbage aphids after 72 h with aphid mortality above 70% which is considered technically effective according to Gonzalez *et al*. (2011). However, a knockdown effect of berry powder was observed on second spray when mortality was 100% after 24 h and became lower after 72 h with 84% (Fig 5.10). Knock down effect is state of intoxication and paralysis of insects before death, some insects may recover from the knockdown effect and survive but some die.

The aphid mortality from field plants ranged from 9 % for water control to 100% for malathion after both 24 h and 72 h (Tables 5.4). Aphid mortalities were significantly different among the treatments. The mortalities from the botanical extracts were generally lower after 24 h with

aqueous berry powder resulting in only 36%. Aphid mortality increased at 72 h; the botanical extracts were above 70% except for aqueous berry powder which remained quite low with 40%. The immature parts (leaf powder and unripe berries) generally resulted in higher mortality than the mature parts (ripe berries) (Fig 5.11). There was a lot of foaming from the fresh berries extracts; foaming could have resulted in uneven spray contact with the leaves and the aphids.

There were varied inconsistent effects of the botanical extracts on the aphids on pot and field plants (Fig 5.12). The typical variation was on berry powder extracts which gave the highest mortality after 72 h on pot plants but gave the lowest mortality on field plants. However water (the negative control) consistently gave significantly high aphid survival and malathion (the positive control) consistently gave the highest aphid mortality.

Figure 5.4: Aphids before (top row) and 48 h after spraying with aqueous leaf powder extract (bottom row)

Figure 5.5 Aphids before (top) and 48 h after spraying with aqueous berry powder extract

Figure 5.6: Aphids before (top row) and 48 h after spraying with aqueous fresh ripe berries extract (bottom row)

Figure 5.7: Aphids before (top row) and 48 h after spraying with fresh unripe berries extract (bottom row)

Fig. 5.8: Aphids before (top) and no aphids 48 h after spraying with malathion

Figure 5.9: Aphids before and 48 h after spraying with water alone

Table 5.2: Aphid mortality from pot plants Spray 1 (24 h)

Treatment	**Aphid Mortality (24 h)**
Malathion	100 a
Aqueous Leaf Powder (ALP)	100 a
Aqueous Ripe Berries (ARB)	100 a
Aqueous Berry Powder (ABP)	89 b
Aqueous Unripe Berries (AUB)	87 b
Water	11 c
LSD at *P*< 0.05	12.0 *
CV %	8

Table 5.3 Aphid mortality from pot plants Spray 2 (24 h and 72 h)

Treatment	**Aphid Mortality (24 h)**	**Aphid Mortality (72 h)**
Malathion	100 a	100 a
Aqueous Berry Powder (ABP)	100 a	84 b
Aqueous Ripe Berries (ARB)	70 b	78 b
Aqueous Leaf Powder (ALP)	71 bc	75 b
Aqueous Unripe Berries (ARB)	53 c	72 b
Water	13 d	20 c
LSD at *P*< 0.05	15.0 *	14.1 *
CV %	18	14

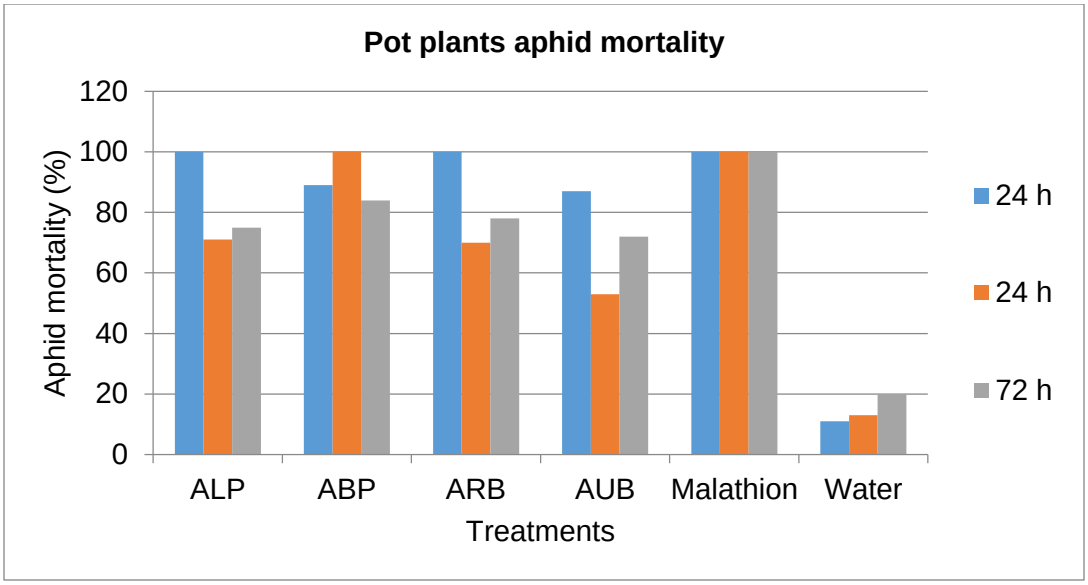

Figure 5.10: Mortality of aphids from pot plants

ALP is aqueous leaf powder, ARB is aqueous berry powder, ARB is aqueous fresh ripe berries and AUB is aqueous fresh unripe berries

Table 5.4: Aphid mortality from field plants (24 h and 72 h)

Treatment	**Aphid Mortality (24 h)**	**Aphid Mortality (72 h)**
Malathion	100 a	100 a
Aqueous Leaf Powder	69 b	86 ab
Aqueous Unripe Berries	61 bc	89 ab
Aqueous Ripe Berries	52 cd	71 b
Aqueous Berry Powder	36 d	40 c
Water	10 e	9 d
LSD at $P < 0.05$	16.8 *	20.3 *
CV %	20	20

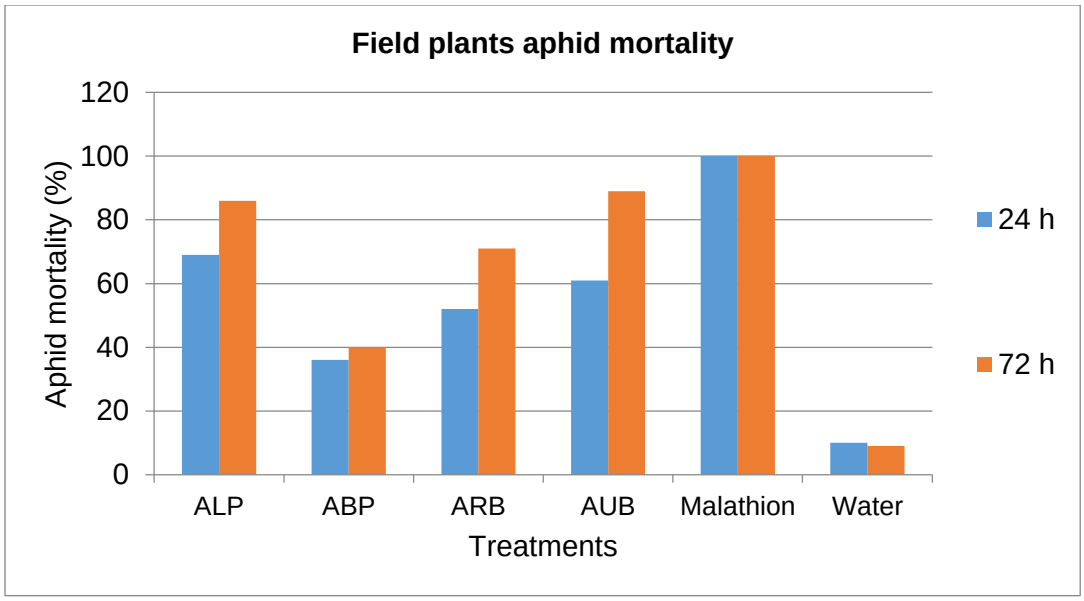

Figure 5.11: Mortality of aphids from field plants

ALP is aqueous leaf powder, ARB is aqueous berry powder, ARB is aqueous fresh ripe berries and AUB is aqueous fresh unripe berries

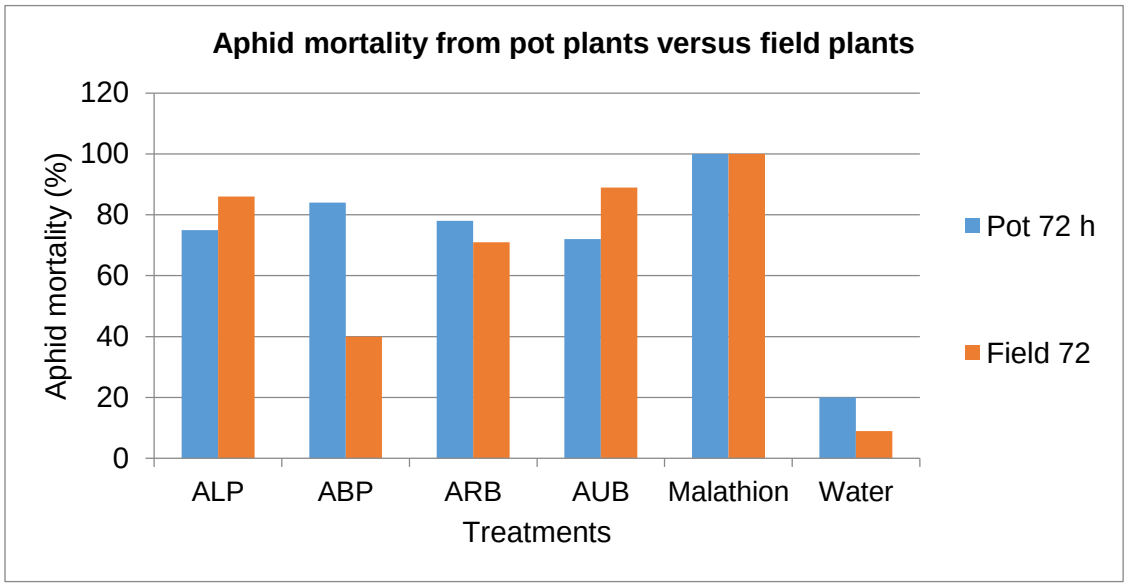

Figure 5.12: Varied effects of the botanical extracts on mortality of aphids from pot and field plants after 72 h

ALP is aqueous leaf powder, ARB is aqueous berry powder, ARB is aqueous fresh ripe berries and AUB is aqueous fresh unripe berries

5.4 Discussion

The results from the plant assays showed that aqueous extracts from *S. panduriforme* were effective against aphids (with above 70 % mortality) but with varied effects. Botanicals are regarded as technically effective against insect pests when they result in mortality of more than 70% (Gonzalez *et al.* 2011). The extracts from aqueous leaf powder, aqueous fresh unripe berries, and aqueous fresh ripe berries consistently resulted in mortalities above 70% both in the field and in pots. The aqueous berry powder extract however gave inconsistent mortality. The reasons for inconsistent effects from the berry powder could be related to incomplete extraction of the active compounds which may not always be the case from batch to batch. According to Sasidharan *et al*. (2011) natural plant materials especially dried materials may contain significant levels of strongly binding components that may compromise bioactivities. The dried parts may need to be treated in a way that ensures compounds of interest are efficiently liberated into solution using HPLC with guard columns for extraction and separation of bioactive compounds. The mortalities were highest on pot plants (simulated use) and lowest on field plants (actual use). Variation in performance of botanical extracts even when prepared under similar conditions has been cited as one of the challenges of using botanical insecticides (Isman 2008).

The plant assays from the pot and field trials however showed that *S. panduriforme* has aphicidal effects from the leaf powder, fresh unripe berries and fresh berries. This is also in agreement with Matu (2008) and Chowanski *et al.* (2016) who reported that bioactive compounds from most Solanaceae plants have insecticidal properties. However, formation of foam from the fresh berries extracts was a challenge during spraying. Foaming is an indication of presence of saponins (Sasidharan *et al*. 2011). The bubbles from the foams increased the droplet size, resulting in uneven spray distribution which influenced the quality of spray. The size of spray droplet is crucial to good pesticide distribution on the plant and insect surfaces (Dobson *et al*. 2002). Small to medium sized droplets generally give good coverage, thus formation of foam resulted in bigger droplets which are might have influenced the effectiveness of the compounds. This could be the reason why the mortality from ripe berries was as low as 36 % and 40 % on field plants. Most botanicals are contact insecticides which require good wetting to be effective (Townsend 2012), however most brassicas have waxy leaves which result in spray droplets bouncing and rolling off the leaves (Dobson *et al*. 2002). This could also contribute to the variation in effectiveness of the extracts. The presence of saponins from the

fresh fruit extracts (Sasidharan *et al*. 2011) and the waxy nature of brassica plants may affect contact and effectiveness of the extracts.

The results from this research are generally in agreement with the findings reported by other researchers. Previous studies indicated that effectiveness of botanicals varied with condition of application and plant part used (Gonzalez *et al.* 2011; Bahar *et al*. 2007). Bahar *et al.* (2007) showed that extracts from the same plant perform differently under field, net-house and laboratory conditions on aphids infesting yard long beans due to mode of application and environment related factors. The most effective aqueous extracts from *S*. *panduriforme* were aqueous leaf powder and aqueous unripe berries though the aqueous berry powder was also effective against aphids on brassica plants under field conditions.

The farmers may use any of the plant parts to control aphids, the aqueous extracts are relatively cheap to prepare and can be sprayed directly on to brassica plants with minimum processing. Dried plant parts which are not perishable like fresh plant materials may be conveniently used to control the aphids in times when fresh parts are not available. For the dried plant extracts to be used effectively, studies on storage conditions to identify how long they remain effective are necessary. It has been suggested that for botanicals to be easily adopted by farmers for use in integrated pest management, they should be used with limited processing (Stevenson *et al.* 2012). Fresh extracts have limited processing as they can be picked and used directly, thus the effective fresh unripe berries may be used when the berries are abundant and the ripe berries may be used in powdered form especially during the off season. The plant may offer all year round protection against aphids on brassicas, as long as the farmers are fully informed on how best they can use the plant parts.

Aqueous and alcohol extracts from plants have been used by other researchers resulting in high mortality of insects (diamond back moth, cabbage aphid, bean aphids and sugarcane woolly aphid (Bahar *et al.* 2007, Baidoo & Adam 2012; Chiffele *et al.* 2009; Mekuaninte *et al.* 2011; Ntonifor *et al.* 2010; Wabale & Kharde 2010). The need to use superior extraction techniques and equipment (Soxhlet, HPLC, solvent-assisted) and the challenges associated with acquisition of alcohol which might be expensive and unavailable, leaves *S. panduriforme* aqueous leaf powder extracts as a better choice for farmers to use for control of the cabbage aphid in their fields. Traditionally, aqueous extracts have been used for insecticidal, veterinary and medicinal purposes (Kareru *et al*. 2013). Resource poor farmers can be advised to harvest

and cheaply prepare botanical extracts from *S. panduriforme* plant parts using water to control problem insect pests like the cabbage aphid. This plant can be easily harvested with care from homesteads, grazing areas, abandoned fields and roadsides.

5.5 Conclusion

The plant assays showed that all the tested plant parts of *S. panduriforme* (young leaf powder, berry powder, fresh ripe berries and fresh unripe berries) have aphicidal effects on brassica plants but vary in effectiveness. Leaf powder gave relatively higher mortalities than berry powder, fresh ripe berries and fresh unripe berries, but any of the plant part may be used to control aphids. The positive control (malathion) gave highest mortality and the negative control (water only) gave lowest mortality.

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CHAPTER 6: QUALITATIVE PHYTOCHEMICAL ANALYSIS OF THE LEAVES AND BERRIES OF *SOLANUM PANDURIFORME*

6.1 Introduction

The botanical importance of any plant is due to the presence of phytochemicals, a term used to describe the large number of secondary metabolic compounds found in plants, such as alkaloids, glycosides, phenols, flavonoids, saponins and tannins. These compounds are concentrated in the various parts of plants, namely the fruits, roots, seeds, bark and leaves (Kumar *et al*. 2011; Singh & Chauhan 2014). The identification of the bioactive compounds has been emphasised to enable synthesis of commercial botanical insecticides that can be adopted by farmers (Chowanski *et al.* 2016; Prakash *et al.* 2008). The knowledge of the chemical constituents of the plants is important for the plant extracts to be used effectively as insecticides for plant protection (Raja & Sama 2012). The extracts from different plants contain various bioactive compounds or phytochemicals with different polarities and these need to be separated and standardised for use (Sasidharan *et al*. 2011; Tiwari *et al*. 2011). However separation and determination of the most bioactive compound still remains a challenge because the compounds have to be purified using a combination of several techniques and various purification methods to obtain, isolate and identify pure bioactive compounds or group of compounds (Handa *et al*. 2008; Sasidharan *et al.* 2011; Yang *et al*. 2004). Natural products are frequently isolated following the evaluation of a crude extract in a biological assay in order to fully characterise the active entity. The bioactive compounds or phytochemicals are then analysed and identified using non-chromatography and chromatography separation techniques (Sasidharan *et al*. 2011).

Non-chromatographic techniques to facilitate the identification of bioactive compounds include immunoassays, phytochemical screening assays, and Fourier-transform infrared spectroscopy (FTIR) (Prashanth & Krishnaiah 2014; Sasidharan *et al*. 2011; Singh & Chauhan 2014). When phytochemical screenings assays are used to identify the compounds, the presence of bioactive compounds is confirmed based on tests of colouration and precipitation. Phytochemical screening assays are simple, quick, and inexpensive procedures that give quick answers to the various types of phytochemicals in a mixture; they are an important tool in bioactive compound analysis (Sasidharan *et al*. 2011). The phytochemical screening research allows to determine qualitatively the main groups of chemical constituents present in a plant. This screening can

guide the subsequent extraction and / or fractionation of extracts for the isolation of groups of interest (Santana *et al.* 2012).

Chromatography techniques that are used to obtain and identify pure compounds include thin layer chromatography (TLC), column chromatography and high performance liquid chromatography (HPLC) (Sasidharan *et al*. 2011). The biologically active compound is often present only as a minor component in the extract. HPLC may be used to rapidly process multi-component samples on both an analytical and preparative scale. Purification of the compound of interest using HPLC is a process of separating or extracting the target compound, in this case it is the compound with aphicidal activities from other structurally related compounds or contaminants. Each compound should have a characteristic peak under certain chromatographic conditions. Depending on what needs to be separated and how closely related the samples are, the chromatographer may choose the conditions, such as the proper mobile phase, flow rate, suitable detectors and columns to get an optimum separation. Identification of pure compounds is based on the comparison of mass spectra and retention indices with published or known results and where possible, with reliable known true compounds (Sajfrtová *et al.* 2013; Sasidharan *et al*. 2011).

Finding new and effective pharmaceuticals and botanicals involves searching for plant substances that are capable for being used to develop new drugs and insecticides against catastrophic illnesses and insect pests (Santana *et al.* 2012). The structural elucidation of bioactive molecules are important for development of new drugs and insecticides. Initial phytochemical screening and further isolation, purification and identification of the molecules have become a major breakthrough with the development of new methods of chromatography and spectroscopy which are expensive, requiring intense analytical procedures; the establishment of new and more effective bioassays is also essential to support bio-discovery programs (Santana *et al.* 2012). The plant extracts fractionation and compounds identification requires subjecting test plant materials to successive extractions with HPLC grade solvents in a closed container, in the absence of light and following correct extraction times.

Considering that *Solanum panduriforme* has been identified by farmers as an insecticidal plant (Stevenson *et al*. 2012) and that bioassays and plant assays carried out in the current research indicated the aphicidal activities of the plant, initial phytochemical analysis of the leaves and berries of *S. panduriforme* was carried out to identify the bioactive compounds with aphicidal

activities. The compounds (alkaloids, flavonoids, phenolics and saponins) are known to have aphicidal effects through acute toxicity, disturbed settling and reduced reproductive capacity (Chowański *et al.* 2016; Golawaska *et al.* 2008a & 2008b). The aim of this research was to carry out initial qualitative phytochemical analysis for aqueous and ethanol extracts from young leaves, ripe berries and unripe berries of *S. panduriforme* which were evaluated in the current research for effectiveness against the cabbage aphid, *Brevicoryne brassicae*. The objectives were to investigate the presence or absence of alkaloids, flavonoids, phenolic compounds and saponins in aqueous and ethanol extracts from young leaves, unripe berries and ripe berries of *S. panduriforme*.

6. 2 Materials and methods

6. 2. 1 Extraction procedures

The aqueous and ethanol extraction techniques using the maceration and homogenisation procedures were used. Water and 60% ethanol were used as the solvents. The rates for extraction were 1:4 w/v for the fresh berries (fresh ripe and fresh unripe) and 1:40 w/v for the powders (leaf powder and ripe berry powder).

6. 2. 2 Phytochemical group analysis

Analytical grade chemicals and reagents were used to detect presence or absence of four chemical groups (alkaloids, flavonoids, phenolic compounds and saponin) thought to have aphicidal effects. Standard methods were used to examine the reactions based on colour changes and precipitation (Kumar *et al*. 2011; Prashanth & Krishnaiah 2014; Sasidharan *et al*. 2011). The tests were carried out once to determine the presence of active phytochemicals alkaloids, phenolic compound, flavonoids and saponins according to the following procedures.

6. 2 .2 .1 Test for alkaloids

To each of the aqueous and 60% ethanol extracts, dilute hydrochloric acid was added, shaken well and filtered. Wagner's reagent test was performed. A few drops of Wagner's reagent were added to 2 ml filtrate in a test tube. Formation of reddish brown precipitate indicated presence of alkaloids.

6. 2. 2. 2 Test for flavonoids

The lead acetate test was carried out. To the aqueous and ethanol extract filtrates, a few drops of lead acetate solution were added. Formation of a yellow precipitate indicated presence of flavonoids.

6. 2. 2. 3 Test for phenolic compounds and tannins

The ferric chloride test was used. To 2 ml of the aqueous and ethanol extract solutions, 5% ferric chloride solution was added. Formation of blue, green or violet colour indicated presence of phenolic compounds.

6. 2. 2 .4 Test for saponins

The froth test was used. The aqueous and ethanol extracts were diluted with distilled water and shaken in a graduated cylinder for 5 min. Formation of a persistent layer of foam indicated presence of saponins.

6. 3 Results

The results of the qualitative phytochemical analysis on the aqueous and ethanol extracts from the young leaves, ripe berries and unripe berries of *Solanum panduriforme* indicated variable presence of alkaloids, flavonoids, phenolic compounds and saponins (Table 6.1)

Alkaloids were present in all the aqueous and ethanol berry extracts (ripe and unripe berries). The test for flavonoids indicated the plant contains flavonoids in aqueous leaf extract and ripe berry extract but absent in the aqueous unripe berry extract. Flavonoids are also present in all the ethanol extracts. The results indicated that phenolic compounds were present in all the aqueous extracts and all the ethanol berry extracts but absent in the ethanol leaf powder extract. The saponins test indicated persistent foaming from the berries extracts indicating presence of saponins in berries and absent in the leaf powders of both solvents (Fig 6.1). More foaming was observed in the fresh berry extracts than the berry powder extract.

Table 6.1: Presence of phytochemicals from leaves and berries of *S. panduriforme*

Plant material	Aqueous extract	Ethanol (60 %) extract
Leaf powder	Flavonoids	Flavonoids
	Phenols	-
Berry powder	Alkaloids	Alkaloids
	Flavonoids	Flavonoids
	Phenols	Phenols
	Saponin	Saponin
Fresh ripe berries	Alkaloids	Alkaloids
	Flavonoids	Flavonoids
	Phenols	Phenols
	Saponin	Saponin
Fresh Unripe berries	Alkaloids	Alkaloids
	-	Flavonoids
	Phenols	Phenols
	Saponin	Saponin

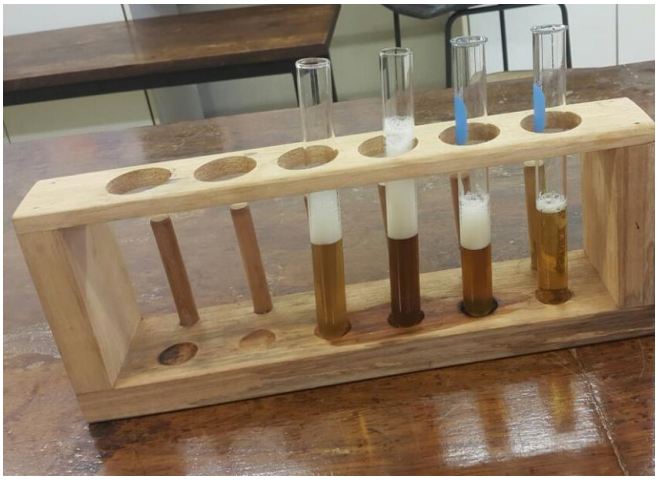

Figure 6.1 The Saponins test showing presence of saponins in the aqueous extracts (Left – Right: fresh unripe berries, fresh ripe berries, ripe berry powder, and leaf powder

The aqueous extract of *S. panduriforme* leaves contained flavonoids and phenols and the ethanol extract contained flavonoids only. The aqueous and ethanol extracts of the ripe berries and the ethanol extract of the unripe berries contain alkaloids, flavonoids, phenols and saponins

though foaming for presence of saponins was not much in the dried berries (Fig. 6.1). The aqueous extracts of the unripe berries contained alkaloids, phenols and saponins and do not contain flavonoids which are found in the ripe berries.

6. 4 Discussion

Alkaloids, flavonoids and phenolic compounds have all been reported to be bioactive against aphids (Chowański *et al.* 2016; Habimana & Hakizayezu 2014). Many alkaloids act as intoxicants and feeding deterrents and are used in crop protection strategies. An alkaloid known as veratrin is the active ingredient for a botanical insecticide commonly sold under the trade names 'Red Devil' and 'Natural Guard', which is developed from sabadilla lily (*Schoenocaulon officinale*), a tropical lily that grows in Central and South America (Dubey *et al.* 2010). Alkaloids have a deterrence and non-preference effect as reported in research on seed predators and seed dispersers by Cipollini & Levey (1997)The research indicated seed dispersers' preference of low-glycoalkaloid *Solanum americanum* fruits and deterrence from ripe *Solanum carolinense* fruits typically with high glycoalkaloid levels. Alkaloid based extracts used as insect deterrents, when applied on plants, can deter insects from feeding thus protecting them from infestation. Alkaloids extracted from chilli were also reported as effective against the cabbage aphid *B. brassicae* (Habimana & Hakizayezu 2014).

Phenolic compounds have antioxidant and insect repellent properties and when ingested by insects decrease growth and development (Chowański *et al.* 2016; Matu 2008). In this qualitative analysis, all the extracts except ethanol leaf powder showed presence of phenols. Phenols have an astringent taste that is repellent to insects. Presence of phenolic compounds in plants reduces the quality and quantity of ingested food by aphids and results in lower fecundity and slower growth of the aphid populations (Golawaska *et al.* 2008a). Phenolic compounds were reported to have negative effects against pea aphids, pea plants with high phenols were resistant to aphids as a result of synergistic effects on aphid behaviour, physiology and metabolism (Golawaska *et al*. 2008b). Lines of alfafa with low levels of phenolic compounds were also found to be better hosts for pea aphid populations. In this research, the aqueous and ethanol berry extracts showed presence of phenols and these could be investigated further for possible use in plant protection programmes.

Both aqueous and ethanol extracted neem leaf and grape leaf extracts were also reported to contain flavonoids (Prashanth & Krishnaiah 2014), similarly leaf aqueous leaf powder contains

flavonoids. Neem leaf extracts are reportedly effective against aphids and are used by farmers to control cocoa insects (Olabninri 2013). In this research, the ripe berries had more persistent foaming indicating presence of saponins. Saponins have been reported to be ineffective against aphids (Habimana & Hakizayezu 2014).

Aqueous and ethanol extracts from *S. panduriforme* contain compounds that are known to be active against aphids. The leaves of *S. panduriforme* contain fewer compounds (flavonoids and phenols) than the berries which contained more (alkaloids, flavonoids, phenols and saponins). Aqueous and ethanol neem fruit extracts were also found to contain more phytochemicals than the leaf extracts, the dried leaf extract typically did not contain saponin and alkaloids (Olabinri *et al*. 2013). However Prashanth & Krishnaiah (2014) found saponin in the aqueous and ethanol leaf extracts of neem. The variation could be due to incomplete extraction when dried plant parts are used. According to Sasidharan *et al*. (2011) dried plant materials need to be treated in a way that ensures compounds of interest are efficiently liberated into solution by using the most appropriate solvents and extraction technique that analyse individual extract samples.

More often the desired effects of bioactive compounds are due to a mixture of chemicals with synergistic effects (McLaughlin *et al*. 1998; Chowański *et al.* 2016). Plant extracts contain various substances that may act synergistically and the relative components of the compounds may vary from batch to batch (Raja & Sama 2012). The qualitative phytochemical analysis showed presence of a mixture of compounds from berries and leaves of *S. panduriforme* that resulted in varied effects on the cabbage aphid (*B. brassicae).* The aqueous and ethanol extracts of neem leaves also indicated presence of many phytochemicals which included flavonoids, alkaloids, glycosides, phenolic compounds and tannins (Singh & Chauhan 2014). Neem has been widely used to control aphids in many crops which include brassicas and cocoa plants (Lajeunesse 2001; Pavela 2009; Varela *et al.* 2003).

6. 5 Conclusion

The phytochemical analysis was positive for all the bioactive compounds tested. The results indicated that *S. panduriforme* contains alkaloids, flavonoids, phenolic compounds and saponins from aqueous and ethanol extracts; the plant has insecticidal properties from both the leaves and the berries. The results from this study indicate that the plant has aphicidal activities from the young leaves, fresh unripe berries and the ripe berries both dried and fresh.

The presence phytochemical components in *S. panduriforme* need further isolation to determine specific compounds and their specific effects on aphids. It is important to identify and isolate specific alkaloids, flavonoids and phenolic compounds from *S. panduriforme* that are effective against aphids and to identify their effects on aphids (whether acute toxicity, deterrence or disturbed growth and reproduction). The results from Chapters 4 and Chapter 5 of this study show that *S. panduriforme* can be considered as important potential botanical for further research and justify its inclusion as an insecticidal plant for integrated pest management. More work is required on how best and easily the compounds from *S. panduriforme* can be incorporated into integrated insect management (IPM) programmes with minimum processing for the benefit of farmers. Not much work has been done to guide its usefulness as an aphicide by farmers and its effects on non-targets have not been identified.

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CHAPTER 7 SUMMARY OF THE RESEARCH

7. 1 Research background

Management of insect pests using synthetic insecticides has health, economic and ecological challenges associated with human and environmental problems (Buss & Park- Brown 2009, Stevenson & Bellman 2016). This is a concern especially to small scale and organic farmers who have limited resouces (Stevenson & Bellman 2016). Botanical insecticides have been suggested as better alternatives to synthetic insecticides; they are reportedly cheaper and less dangerous to humans and to the environment (El-Wakeil 2014). A number of plants have been identified for use as insect toxicants, repellents and antifeedants but there has been little success in their use in plant protection (Isman 2006; El-Walkeil 2014). Chemical standardization based on active ingredients has not been done on most plants. This has resulted in less adoption of pesticidal plants use by farmers; they experience variations on botanical insecticides effectiveness when they use plants to control target insect pests (Isman 2006; Stevenson *et al*. 2012). Non-standardized extraction of bioactive compounds may also cause degradation of the phytochemicals, resulting in variations on effectiveness of botanicals insecticides (Tiwari *et al*. 2011). The quality and composition of the plant extracts may also vary due to natural make-up of the plants or use of plant materials from different harvestings or different locations. Efforts to produce consistent extract batches as much as possible are critical; the efforts include, but not limited to, development of most appropriate harvesting methods and use of best extraction processes and techniques that can be followed by users of the botanical extracts; be it for medicinal, veterinary or plant protection purposes.

According to Miresmailli & Isman (2014), to ensure viable use and consistent effectiveness of botanical insecticides factors like resource availability, extraction and formulation techniques, innovative application technologies and production cost should be considered. The cost of plant protection using synthetic insecticides has been reported to be always higher than using botanical extracts (Stevenson & Belman 2016). When insect pests of cabbages were controlled using the crude extracts of *Ageratum conyzoides*, *Chromolaena odorata* and *Synedrella nodiflora*; the cost-to-benefit ratio of the botanicals extracted following basic extraction steps, using appropriate extraction rates and solvents and correctly applied was higher than the synthetic insecticide emamectin benzoate. The costs that were considered were based on material and labor costs versus the revenue derived from the marketable yield of cabbages. Cheaply prepared aqueous extracts of *Tephrosia vogelii* or *Tithonia diversifolia* used to control

insects also resulted in better bean yields as compared to the expensive synthetic insecticide Karate, - lambda-cyhalothrin, which was also similar to aqueous *Lippia javanica* or *Vernonia amygdalina* extracts (Mkenda *et al.* 2015).

Studies to examine the bioactivity of the cheaper extracts are very important to enable identification of potential sources of bioactive chemicals with pesticidal activities and to provide suggestions on potential mode of action. Most plants including *Solanum panduriforme* have not been evaluated for their use in insect pest management programmes. This study focused on extraction techniques to enable identification of bioactive chemicals from *S. panduriforme*. Since extraction is the first crucial step in the screening of bioactive compounds, the bioactive compounds of *S. panduriforme* (leaves, ripe berries and unripe berries) were extracted using maceration, homogenisation and solvent assisted extraction techniques and were tested for their effectiveness on the cabbage aphids (*Brevicoryne brassicae*) on brassicas in the laboratory, screen house and in the field.

7.2 General results and discussion

A preliminary evaluation of three concentration levels using leaf dip residual bioassays indicated that the most appropriate ratio for extraction on a weight per volume basis using water and ethanol as the solvents is 10 g per 400ml (1:40 w/v) for powders and 100 g per 400 ml (1:4 w/v) for fresh berries. These ratios resulted in 70% aphid mortality which is regarded as technically effective. For leaf power 10 g is roughly 4 level teaspoons and 10 g berry powder is roughly 3 level teaspoons. The fresh berries weight depended on the size of the berries; for 100 g, 17 medium sized berries are needed, 10 large berries to 13 medium berries are need for 100 g unripe berries. There has not been much literature on exact amounts for ripe berries generally used by the few farmers who have been reportedly used the plant for plant protection let alone the powders which have not been used at all. Other researchers have used ratios ranging from 1: 2 w/v, 1:5 w/v, and 1: 10 w/v up to 1:25 w/v for dry material to achieve effective control of insects (Ntonifor *et al.* 2010; Tiwari *et al* 2011). In this research these ratios were too high resulting in a thick mixture of leaf powder and berry powder which could not be used to spray the plants.

The three bioassay techniques showed that the aqueous and ethanol extracts from *S. panduriforme* leaves and berries have aphicidal effects on the cabbage aphid. However the cabbage leaf and aphid dip bioassays had challenges of extract to leaf surface contact which

was not observed when mustard rape plants were used for the preliminary leaf dip residual bioassays. This is because the cabbage leaf has higher wax levels than the mustard rape leaves (Dobson *et al.* 2002). Results from the subsequent bioassays showed that the immature plant parts (leaf powder and fresh unripe berries) are generally more effective with acute toxicity effects than the mature plant parts (berry powder and fresh ripe berries). The effects of the extracts on aphids (acute toxicity, non-preference and disturbed settling) were observed on aqueous extracts in choice and no choice bioassays. The immature parts (leaf powder) resulted in acute toxicity (higher mortality) and the mature parts (ripe berries) resulted in more of deterrence effects (disturbed settling and non-preference). Other effects from the aqueous extracts which were observed included disturbed reproduction; nymphs were observed on water control and the ripe berries extracts which generally were ineffective than the unripe berries and the leaf powder. Some adults even formed wings on ripe berries and water, an indication of survival and failing to form established colonies on the treated leaf cuttings with less acute toxicity effects.

Extracts from organic solvents (hexane and diethyl ether) almost resulted in total aphid mortality in the topical bioassays; with hexane extracts being more superior to diethyl ether extracts. Aqueous extracts also resulted in lower mortalities which were however technically effective against aphids with mortality above 70 %. There is a high possibility of reducing the extract concentration from 50mg crude extract per ml of acetone that was used in this study to 25 mg per ml or even less since aphids died immediately after extracts were topically applied on the aphids. This may result in use of lower amounts of botanical plant materials making it very sustainable to use the plant as a botanical to control the aphids on the brassicas. Since this study looked on effectiveness, work on efficacy is crucial to determine the LC 50 and LD 50 rates which may then be used to determine the amounts of material required to control aphids per hectare. Detailed fractionation of the plant extracts to obtain specific bioactive compounds with repetitive rescreening against the aphids is also necessary to come up with the efficacy details of the plant parts. This requires close cooperation of organic chemistry analysts and entomologists. Efficacy evaluation includes the effect on the cabbage aphid; the reliability, duration and consistency of protection; effects on quantity or quality of the yield of treated plants; safety considerations to the crop; effectiveness comparison with accepted practices; compatibility with other crop protection measures; undesirable or unintended side effects, on beneficial and other non-target organisms (FAO 2006).

The aqueous extracts from leaf powder, berry powder, fresh ripe berries and fresh unripe berries tested on artificially infested screen house plants and naturally infested field plants indicated that *S. panduriforme* leaves and berries have aphicidal effects. Leaf powder, fresh ripe berries and fresh unripe berries consistently resulted in effectiveness of above 70% aphid mortality from both the screen house and field plants assays.Berry powder was inconsistent with a low 40% mortality from field plant assays and high mortality of above 70% from the screen house plant assays. As mentioned earlier the variation in botanical extracts effectiveness even when prepared by same process is one of the challenges for their adoption by farmers.

The phytochemical evaluation of the leaves and berries indicated presence of flavonoids in the leaf powder extract (both aqueous and ethanol). All the tested bioactive compounds (alkaloids, flavonoids, phenolic compounds and saponin) were present in the berry extracts (powdered, fresh ripe and fresh unripe) though the aqueous fresh unripe berries did not show presence of flavonoids. Absence of the flavonoids in the unripe berries could have contributed to positive synergistic effects from less compounds when compared to more compounds which were present in the ripe berries. The consistent effectiveness of leaf powder in most bioassays and in the plant assays was most likely due to the presence of flavonoids which are known to have acute toxicity effects on aphids. Flavonoids were found to be active aphicides against the woolly aphid (Atteyat 2012; Chowanski *et al.* 2016); when the concentration of the the flavonoids is increased nymph mortality also increases. The leaf powder from *S. panduriforme* caused high aphid mortality in the bioassays (Chapter 4) and the plant assays (Chapter 5) carried out in this research. The higher aphids' mortality from the leaf powder extracts is most likely due to the acute toxicity effect from the flavonoids. Aqueous leaf powder also resulted in higher inhibition of aphids settling in the no choice bioassays. There was more disturbed settling from the leaf powder. It was also confirmed by other authors that different flavonoids modulate the feeding and oviposition behaviour of insects (Atteyat, 2012; Sallam *et al.* 2009). Flavonoids may not cause insect death for several days but ingestion of small quantities may result in insects becoming inactive and stop feeding (Sallam *et al.* 2009). The residual insecticidal activity of botanical extracts may be for up to seven days, depending on both aphid species and concentrations (Sallam *et al.* 2009). Both aqueous and ethanol extracted neem leaf and grape leaf extracts were also reported to contain flavonoids (Prashanth & Krishnaiah 2014). Neem leaf extracts are reportedly effective against aphids and are used by farmers to control cocoa insects (Olabninri 2013).

The effectiveness of the berries (both ripe and unripe) could have been due to the presence of alkaloids which have more of deterrence and anti-feedendant effects, however the presence of saponin in the fresh berries extract which was generally less effective against aphids could have contributed to the lower mortalities from the fresh berries. Because of the complex chemical composition; botanical insecticides have multiple modes of action and chances of insects developing resistance from the many different compounds are regarded as minimum (Chowanski *et al*. 2016). This has been cited as one of the advantages of botanical insecticides over synthetic insecticides.

In this research, there was variation in effectiveness of the berries. The fresh unripe berries are reported as highly effective against aphids' mortality in the preliminary bioassays and topical bioassays in Chapter 4, berry powder is reported effective on pot plants assays but less effective on field plants assays in Chapter 5 of the current research. The quality and quantity of alkaloids in berries of *S. panduriforme* can be researched further to find out how best the alkaloids can be isolated from the ripe berries and unripe berries for use as an active ingredient in the synthesis of a commercial or home-made botanical insecticide in a similar manner to other successfully used botanicals like sabadilla and neem. The fresh berries are abundant in areas where the plant grows especially in abandoned fields and grazing areas and may be put into better use. The ripe berries of *S. panduriforme* are big and may be a good source of alkaloids for commercial botanical insecticide production; it has been reported that because of their small size, *S. nigrum* immature fruits which have a high content of alkaloids are not a promising source of alkaloids for commercial botanical insecticides (Hammami *et al.* 2011). In some countries *S. panduriforme* is considered as an invasive weed which need to be destroyed (Masingi *et al.* 2008). Utilisation of the berries for botanical insecticides might change the plant status from invasive weed to a useful botanical plant. According to Chowański *et al.* (2016), the effects of Solanaceae secondary metabolites on insects can be disturbed growth, disturbed metabolism, disturbed reproduction, behaviour change and death due to anti-feedence, midgut malfunction and acute toxicity. *Solanum panduriforme* has a high possibility of being used to suppress aphid populations on brassicas since some of these effects were observed in this study.

7.3 Conclusion

This research (bioassays, plant assays, and phytochemical analysis) indicated that *S. panduriforme* is a useful aphicidal plant, the leaves and the berries can be put to good use in integrated pest management (IPM) programmes. It has the ideal properties of insecticidal plants which include wide distribution and other additional uses for veterinary and medicinal purposes (Silva-Aguayo 2009). *Solanum panduriforme* is abundant and common as a weed, around houses (Fig 7.1), in overgrazed grassland and on roadsides (Lusweti *et al.* 2011; Matu 2008). The flowering time is mainly from November to March thus the leaves and fruits can be easily collected for use throughout the year. Both the mature and immature plant parts have aphicidal properties when used as aqueous extracts and organic solvents (ethanol, hexane and diethyl ether) extracts.

Figure 7.1 Mature and immature *S. panduriforme* plants growing around homesteads

This study generated basic information on the use of *Solanum panduriforme* as a pesticidal plant. The methods for extracting aqueous and organic solvents (ethanol, hexane and diethyl ether) bioactive compounds using the homogenization extraction, maceration extraction and sequential separation techniques are highlighted. The main groups of bioactive compounds from the leaves and berries were confirmed with an indication on how much can be obtained from the respective parts. Leaf powder which was consistently effective had the highest total bioactive compounds in mg. Data was generated on the effects of *Solanum panduriforme*

aqueous and ethanol extracts from leaves and berries and the dried crude extracts from hexane and diethyl ether on aphids' mortality under laboratory and field conditions. The data is valuable for use as a starting point to carry out toxicological and efficacy studies of *Solanum panduriforme* extracts on aphid mortality. Information from this study is crucial to the development and standardization of *Solanum panduriforme* based botanical insecticides. These results also help to give assurance to farmers that pesticidal plants are as effective as synthetic insecticides. This will be of immense and tremendous benefit to integrated pest management and sustainable vegetable production.

The extracts from the leaves and fruits of *S. panduriforme* did not have the same effects on the mortality of the cabbage aphid (*B. brassicae*) on brassicas but they were all effective in some way. The null hypothesis which states that; "There is no difference in mortality of the cabage aphid, *Brevicoryne brassicae* treated with aqueous extracts, ethanol extracts, and solvent assisted extracts (hexane and diethyl ether) extracts from the dried leaves, dried ripe berries, fresh ripe berries and fresh unripe berries of *Solanum panduriforme*, the extracts contain the same bioactive compounds" is rejected. The alternate hypothesis which states that; "There is a difference in mortality of the cabage aphid, *Brevicoryne brassicae* treated with aqueous extracts, ethanol extracts, and solvent assisted extracts (hexane and diethyl ether) extracts from the dried leaves, dried ripe berries, fresh ripe berries and fresh unripe berries of *Solanum panduriforme*, the extracts do not contain the same bioactive compounds" is accepted

7.4 Recommendations

1. Aqueous extracts of immature plant parts (leaf powder and unripe berries) can be used directly by farmers to control the cabbage aphid (*B. brassicae)* on brassica plants at a rate of 1:4 w/v fresh unripe berries and 1:40 w/v dried leaf powder full cover spraying.
2. The hexane, diethyl ether and ethanol extracts of *S. panduriforme* need further analysis and evaluation for their effects on growth and development of the cabbage aphid *B. brassicae* for their future use in *S. panduriforme* botanical insecticides formulations.
3. Fractionation, chemical characterisation and structural identification of specific compounds should be done to enable development of a commercial based aphicide for use by farmers who can afford it. The berries of *S. panduriforme* are big and they contain alkaloids which are toxic and cause death of insects in very low doses. (LC 50 is 5 - 40 mg/L on sugarcane aphid and 0.1–1.6 mg/mL of diet on green peach aphid). Alkaloids are the most common biologically active compounds within the Solanaceae family, various alkaloids result in various effects on insects. If “Red devil has been developed from Sabadilla lily in South America, there is a possibility that “Yellow evil” can also be developed from *S. panduriforme.*
4. Evaluation of the effect of *S. panduriforme* extracts on other aphid species, other brassica insect pest and beneficial insects is needed to determine suitability of the extracts in integrated pest management approaches.
5. Participatory research by farmers can lead to acceptance and adoption of use of the plant; this can be done through on-farm research field trials by varying extract concentration and spraying intervals as equally important factors for effective insect pest management.

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