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Insecticidal activity of a native Australian tobacco, *Nicotiana megalosiphon* Van Heurck & Muell. Arg. (Solanales: Solanaceae) against key insect pests of brassicas

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ABSTRACT

Concerns over the harmful effects of synthetic insecticides have stimulated interest in alternative pest management tactics including botanical insecticides that provide novel modes of action against pests that have developed resistance against synthetic insecticides. Much work has been conducted using plants native to various developing countries but the Australian flora has been little explored as a source of novel plant protection treatments. Here we report potent insecticidal properties of the Australian plant, native tobacco *Nicotiana megalosiphon* against three key pests of brassicas: the diamondback moth (*Plutella xylostella*), cabbage aphid (*Brevicoryne brassicae*) and the green peach aphid (*Myzus persicae*). Aqueous extracts (1%, 5% and 10% w/v) of *N. megalosiphon* gave up to 100% control of *P. xylostella*; better than the recommended rate of tau-fluvalinate (a synthetic pyrethroid) or equivalent extracts of a second Australian native plant, *Mentha satureioides.* Treatment with *N. megalosiphon* also gave 100% control of *B. brassicae* at the highest two concentrations, and of *M. persicae* at the highest concentration. In a second study using F1 *P. xylostella* survivors of tau-fluvalinate treatment, the highest two rates of *N. megalosiphon* caused 100% mortality though efficacy of tau-fluvalinate was eroded compared with use on the previous generation. Chemical analysis of *N. megalosiphon* recorded the presence of anabasine at 5.2 µg/ml in water extracts of leaves and 59.4 µg/ml in methanol extracts of leaves. While these concentrations were low particularly for the water extracts they might nevertheless explain the biological activity of *N. megalosiphon.*

1. Introduction

Globally, pests consume crops sufficient to feed an additional one billion people (Birch et al., 2011), hence managing them effectively is key to higher crop productivity. The diamondback moth *Plutella xylostella* L. (Lepidoptera: Plutellidae) is the most important pest of brassicas and its successful management presents a daunting task to brassica farmers worldwide (Sarfraz and Keddie, 2005; Charleston et al., 2006a; Furlong et al., 2013; Li et al., 2016). It was the first crop pest to develop resistance to DDT, and has successively developed resistance to all classes of chemical insecticides as well as some strains of insecticides based on toxins produced by the bacterium *Bacillus thuringiensis* (Sarfraz and Keddie, 2005; Grzywacz et al., 2010). Global annual cost of managing diamondback moth and loss in production is estimated at US$ 4-5 billion (Furlong et al., 2013). The cabbage aphid, *Brevicoryne brassicae* L. and the green peach aphid, *Myzus persicae* Sulzer (Hemiptera: Aphididae) also cause substantial damage to brassicas (Severtson et al., 2016). Both the nymphal and adult stages of aphids are phloem feeders (Goggin, 2007). Aphids have short generations due to their ability to reproduce by parthenogenesis (Goggin, 2007). Aphids account for 50% of insect-vectored plant viruses (Ng and Perry, 2004).

Though biological control of the foregoing pests is possible, it rarely proves effective due to late or inadequate build-up of natural enemies, often as a result of synthetic insecticide use (Macharia et al., 2005). Consequently, commercial brassica vegetable production often rely on application of synthetic insecticides (Charleston et al., 2006b). But the use of synthetic insecticides is associated with human health and

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several ecological problems including poisoning, contamination of food commodities, environmental and water pollution, pest resistance and resurgence and elimination of non-target organisms (Williamson et al., 2008; Pimentel, 2009). Attempts to control vegetable brassica pests, especially *P. xylostella,* can lead up to 20 applications of synthetic insecticides within a growing season in some parts of the world (Oliveira et al., 2011).

Botanical insecticides provide an alternative pest management option, especially in situations where synthetic insecticides have proved ineffective due to pest resistance (Koul, 2004). They are often described as benign on beneficial arthropods and the ecosystem due to their non-persistent nature (Dubey et al., 2011; Kedia et al., 2015). Their rapid break-down upon exposure to the atmosphere (Dubey et al., 2011), greatly reduces the risk of residues in food. Botanical insecticides can be selective (Amoabeng et al., 2013), and do not frequently result in insecticidal resistance as often occurs with synthetic insecticides (Isman, 2002; Koul, 2004; Charleston et al., 2006a, b).

Despite the wealth of knowledge of botanical plants native to developing countries, little work has been done on the Australian flora so we evaluated the insecticidal activity of two Australian plants: native tobacco *Nicotiana megalosiphon* Van Heurck & Muell. Arg. (Solanales: Solanaceae) and native Pennyroyal *Mentha satureioides* R.Br. (Lamiales: Lamiaceae). These were selected because congeneric of each are known to have insecticidal properties but no work has been done on these species. *Nicotiana megalosiphon* is found in central and south-eastern Queensland and northern New South Wales (NSW) (Voeks, 2012) whilst *M. satureioides* is widespread in South Australia, NSW, Victoria and Queensland (McIntyre et al., 1995). *N. megalosiphon* is an uncultivated species but has been used as parent in some genetic breeding programs of tobacco due to its high resistance towards several important pathogens (Chacón et al., 2009). Commercial tobacco, *N. tabacum* has four main alkaloids: nicotine, nornicotine, anabasine, and anatabine with nicotine content of 90–95% and the remaining three alkaloids account for 5-10% of the total alkaloid content (Siminszky et al., 2005) but little is known about the chemistry of *N. megalosiphon*. According to Voeks (2012), the plant has nornicotine content of 0.22% and little or no nicotine.

The genus *Mentha* has about 19 species and 13 natural hybrids across the globe (Kumar et al., 2011). *Mentha* is among the common herbs that have been known for medicinal and food flavouring properties since ancient times but its insecticidal properties too have been reported (Fialová et al., 2008; Kumar et al., 2011).

The insecticidal properties of *Nicotiana* and *Mentha* species have been exploited in managing several insect pests of economic importance. For instance, aqueous extract of *N. tabacum* was effective against pests of cabbage including diamondback moth, cabbage aphids and green peach aphid (Rando et al., 2011; Amoabeng et al., 2013). Species of *Mentha* have also been exploited to manage pests based on several modes of action such as repellence, adulticidal contact toxicity, antifeedance against storage pests such as *Sitophilus zeamais*, *S. oryzae* (Coleoptera: Curculionidae), *Tribolium castaneum* (Coleoptera: Tenebrionidae), *Callosobruchus maculates* (Coleoptera: Chrysomelidae) (Kumar et al., 2009, 2011) and onion thrips, *Thrips tabaci* (Thysanoptera: Thripidae) (Koschier et al., 2002). Insecticidal activity of *Mentha* species including *M. arvensis* (Kumar et al., 2009, 2011), *M. longifolia* (Odeyemi et al., 2008), *M. spicata, M. microphylla* (Farghaly et al., 2009), *M. rotundifolia* (Ferraro et al., 2003) and others have been reported. There is, however, no report on the insecticidal activity of *M. satureioides* or *N. megalosiphon*. Accordingly, this study aimed to explore the insecticidal activity of aqueous extracts of *M. satureioides* and *N. megalosiphon* against three important pests of brassicas: *P. xylostella, B. brassicae* and *M. persicae*.

2. Materials and methods

2.1. Insects

Larvae of *P. xylostella,* adult *B. brassicae* and *M. persicae* were collected from an organic cabbage farm close to Orange, New South Wales, Australia (33.3679° S, 149.1314° E) in April 2016. Each insect species was reared separately in multiple cages on potted cabbage plants in a glasshouse at the Charles Sturt University, Orange campus, NSW (33.2465° S, 149.1173° E) at 23 °C ± 5 °C, RH 75% ± 5% and unregulated photoperiod of about 11 h. *P. xylostella* and *B. brassicae* were reared to the 6th generations whilst *M. persicae* reached the 8th generation before they were used for the experiment. The bioassays were performed on larvae of *P. xylostella* and adult *B. brassicae* and *M. persicae*.

2.2. Plants

Seeds of *M. satureioides* and *N. megalosiphon* were donated by Diversity Native Seeds, Coonabarabran, NSW (31.2733°S, 149.2191°E) and sown in May 2016 using proprietary seed raising and cutting mix (Osmocote) obtained at Orange, NSW. Seedlings were transplanted into plastic pots filled with the same potting medium then grown in a separate glasshouse to that used for insect rearing but with the same environmental conditions.

2.3. Experimental design and treatment application

The three herbivores were tested in separate experiments but with the same treatments: aqueous extracts of *M. satureioides* and *N. megalosiphon* at three concentrations (1, 5 and 10% m/v), Yates, Mavrik (7.5 g/L tau-fluvalinate) a synthetic pyrethroid widely used against Lepidoptera and Hemiptera pests in many crops, and tap water as control. Tau-fluvalinate was used at the rate of 9.5 ml/L. This resulted in eight treatments and each was replicated six times. Experimental units consisted of potted cabbage *Brassica oleracea* var. *capitate* cv. Sugarloaf seedlings at six weeks old (about 6 true leaf stage).

2.4. Aqueous extract preparations

Fresh, fully-expanded leaves of *M. satureioides* and *N. megalosiphon* that were about five and half months old were collected and weighed. The quantity of plant material to give the various concentrations for each plant was blended separately using an electric blender. To obtain the various concentrations, 5 g, 25 g and 50 g of fresh leaves were blended and mixed with 500 ml tap water (ambient temperature) to obtain 1%, 5% and 10% concentrations respectively of each of the aqueous plant extracts. Blending speed and duration varied among treatments reflecting the different properties and volumes of plant material but in all cases it extended until no plant fragments were visible to the naked eye. Water was selected as the solvent as it is the cheapest, safest and the most accessible polar solvent for any future use of these plants. Menthol the active constituent in *Mentha* species is considered polar and thus can be dissolved in water. Aqueous extract of *Nicotiana tabacum* was successful against cabbage pests (Amoabeng et al., 2013). Tap water was used so that results would reflect the efficacy that a grower would obtain if preparing a crude extract outside of laboratory conditions. The mixtures were filtered with muslin cloth to obtain particle-free plant extracts and put into 1 L capacity hand sprayer for immediate application after Amoabeng et al. (2013).

2.5. Bioassay of the herbivores

For *P. xylostella*, sixth generation, third instar cohorts (larvae) from glasshouse culture as described above were used. Ten larvae were taken from the rearing cages and put on potted caged cabbage seedling using