Terpene based biopesticides as potential alternatives to synthetic insecticides for control of aphid pests on protected ornamentals

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1   **Terpene based biopesticides as potential alternatives to synthetic insecticides**

2   **for control of aphid pests on protected ornamentals**

3

4   **Highlights**

5   1. Terpene based biopesticides show promise against aphid pests of ornamental crops

6   2. Products tested based on orange oil, *Chenopodium ambrosioides* extract and neem oil

7   3. Against *Myzus persicae*, orange oil gave a similar speed of kill to flonicamid

8

9   **Abstract**

10   Biopesticides based on plant extracts offer a promising alternative to the use of conventional

11   synthetic pesticides. However, biopesticide products must provide acceptable levels of

12   control. To date, few studies have investigated the efficacy of biopesticide products under

13   conditions that reflect commercial practice. Here we report results from three experiments,

14   one completed under glasshouse conditions in 2014 and two completed under polytunnel

15   conditions, in 2015 and 2016, respectively. These experiments tested the efficacy of three

16   terpene based biopesticides used to control two aphid species, peach-potato aphid (*Myzus*

17   *persicae*) and melon and cotton aphid (*Aphis gossypii*), on ornamental crops. The three

18   biopesticide products tested were orange oil (60 grams active ingredient per litre, formulated

19   as a soluble liquid), the essential oil from *Chenopodium ambrosioides* variety nr.

20   *ambrosioides* (16.75% active ingredient, formulated as an oil dispersion) and neem oil (1%

21   active ingredient, formulated as emulsifiable concentrate). The biopesticides tested were

22   applied as foliar sprays using a water volume of 600 l/Ha and all experiments were done at

23   Harper Adams University, Shropshire, UK.

24

25   The biopesticide products tested gave statistically similar levels of control of *M. persicae*

26   populations on pansy plants as the conventional synthetic insecticide flonicamid (500 g/kg

27   active ingredient, formulated as a wettable granule) and spirotetramat (150 g/l active

28   ingredient, formulated as an oil dispersion). All products reduced numbers of aphids by at

29   least 85% during the experimental period. Orange oil also gave a similar speed of kill to

30   flonicamid and was faster acting than spirotetramat, two conventional synthetic insecticides

31   that are widely used to control aphid pests of ornamental crops. Against *A. gossypii* on

32   *Hebe*, orange oil gave similar levels of control (90% reduction in aphid numbers) as

33   flonicamid (98% reduction in aphid numbers), when applied with a spray interval of three

34   days (as per label recommendation). The essential oil from *Chenopodium ambrosioides*

35   variety nr. *ambrosioides* was not as effective as flonicamid but did significantly reduced (80%

36   reduction in aphid numbers) numbers of *A. gossypii* on *Hebe* compared to a water control

37   when applied with a spray interval of five days. Neem oil was not effective against *A.*

38   *gossypii*. Importantly, there was little evidence of any phytoxicity caused by any of the

39   biopesticide products tested. The potential to use these products as part of an Insecticide

40   Resistance Management (IRM) programme are discussed.

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48   **Introduction**

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50   Continued reliance on synthetic pesticides to provide effective control of crop pests, weeds

51   and diseases is under increasing pressure. The number of active ingredients permitted for

52   use in the European Union (EU) has declined from *c.* 1,000 actives in 1993 to around 250 in

53   2011 (Chapman, 2014). This decline is largely the consequence of regulatory changes, e.g.

54   implementation of EU Regulation 1107/2009 and an associated shift from risk to hazard

55   based assessment of pesticide safety in terms of human health and the environment. There

56   are also financial and time constraints on the development of active ingredients. The cost of

57   bringing a new active ingredient to market has increased from $152 million in 1995 to $256

58   million in 2005 (Chapman, 2014) while the time taken to develop and register a new

59   conventional pesticide is now around 10 years (Glare et al., 2012). As a result, while there

60   were around 70 new active ingredients in the development pipeline in 2000, this number had

61   dropped to 28 in 2012 (Chapman, 2014). These challenges facing the industry are

62   compounded by increasing numbers of cases of pesticide resistance, with over 580

63   arthropod species being recorded as having developed resistance globally (Sparks and

64   Nauen, 2015).

65

66   Biopesticides offer a promising alternative to the use of conventional synthetic pesticides

67   due to reduced risk of resistance developing in pest populations, lower development costs,

68   higher target specificity, lower environmental persistence, and generally improved

69   compatibility with biological controls (Copping and Menn, 2000; Chandler et al., 2011;

70   Hubbard et al., 2014; Seiber et al., 2014). Plant essential oils are synthesised through

71   secondary metabolic pathways and have long been recognised to possess insecticidal

72   and/or repellent properties (Regnault-Roger et al., 2012; Isman, 2016). Although plant

73   essential oils are typically complex mixtures, they are often dominated by two or three

74   chemical compounds that can be usually classified into two chemical groups, terpenes or

75   phenylpropanoids (Edris, 2007; Bakkali et al., 2008; Regnault-Roger et al., 2012).

76

77   There are many examples of biopesticides based on terpenes, including orange or citrus

78   oils, essential oil derived from *Chenopodium ambrosioides* variety nr. *ambrosioides*

79   (Chenopodiaceae) and neem extracts. Orange or citrus oils include *d*-limonene as the major

80   component (Hink and Fee, 1986) and are known to be toxic to a wide range of insect pest

81   species (Sheppard 1984; Hink and Fee, 1986; Karr and Coats 1988; Hollingsworth, 2005;

82   Raina et al., 2007). Products based on orange or citrus oils work through contact as well as,

83   in some cases, fumigation action. The essential oil derived from *Chenopodium ambrosioides*

84   variety nr. *ambrosioides* L., consists of a mixture of 14 monoterpenes and is known to be

85   toxic to a range of insect and mite pest species (Chiasson et al., 2004a,b; Cloyd and

86   Chiasson, 2007). Finally, oil extracted from *Azadirachta indica* A. Juss. (neem) seeds

87   includes, as the major compound, azadirachtin A (a triterpene), which has been shown to

88   have antifeedant and repellent properties as well as inducing sterility in insects, such as the

89   peach-potato aphid (*Myzus persicae* (Sulzer)) (Mordue (Luntz) et al., 1996; Chaudhary et al.,

90   2017). Although biopesticides based on plant essential oils have previously been shown to

91   have insecticidal effects, their modes of action are poorly understood. The few studies so far

92   completed suggest that plant essential oils are cytotoxic and/or neurotoxic (Pavela and

93   Benelli, 2016), but further work is required in this area. It is also possible that any insecticidal

94   effects observed are the consequence of synergy between different plant essential oils that

95   have differing modes of action. A review of the biological effects of plant essential oils is

96   provided by Bakkali et al. (2008).

97

98   Around 100 species of aphid are considered to be agricultural pests of a wide range of crops

99   (van Emden and Harrington, 2007). Two of the most important aphid pest species are *M.*

100   *persicae* and the melon and cotton aphid (*Aphis gossypii* Glover) due to the wide range of

101   crops that they may infest and their ability to develop resistance to conventional synthetic

102   insecticides, such as carbamates (e.g. Furk and Hines, 1993; Foster and Blackshaw, 2012),

103   pyrethroids (e.g. Marshall et al., 2012; Foster and Blackshaw, 2012) and neonicotinoids

104   (Bass et al., 2011; Herron and Wilson, 2011).

105

106   Both *M. persicae* and *A. gossypii a*re important pests of ornamental crops (Alford, 2003),

107   where they present a major challenge to growers due to a lack of tolerance to damage and

108   difficulty in achieving effective control using available conventional synthetic insecticides.

109   Despite increasing interest in, and availability of, biopesticides few studies have investigated

110   the efficacy of biopesticide products relative to currently used conventional synthetic

111   insecticides under conditions that reflect commercial practice. This study investigates the

112   potential of three terpene based biopesticides for the control of *M. persicae* and *A. gossypii*

113   on protected ornamental crops.

114

115

116   **Materials and methods**

117

118   *Insects*

119   A population of a single clone of *M. persicae*, known to be resistant to pyrethroid and

120   carbamate insecticides, was maintained on pak choi (*Brassica rapa* L.) seedlings prior to use

121   in experiments. *Aphis gossypii* were collected from a commercial ornamentals nursery and

122   maintained on cotton seedlings (*Gossypium hirsutum* L.) prior to use in experiments. Both

123   aphid populations were maintained in fine mesh insects cages of size 47.5 x 47.5 x 47.5 cm

124   (BugDorm, MegaView Science, Taichung City, Taiwan). Plants were changed each week and

125   fresh plants infested with aphids taken from the discarded plants. Insect cages were placed in

126   controlled environment rooms (Weiss Technik UK Ltd, Ebbw Vale, UK) set to 20°C, 60%

127   relative humidity and 16:8 (light:dark) hours.

128

129   *Plants*

130   Pansy (*Viola* x *wittrockiana* var. Lubega F1 Mix) and *Hebe* 'Purple Pixie' plug plants were

131   planted into 9 cm diameter pots in May in each year of the project. Pansy plants were potted

132   into a peat based growing medium (M3 Pot/Bedding Compost, Levington, Frimley, UK) while

133   *Hebe* plants were potted into a soil based growing medium (John Innes No. 2, J Arthur

134   Bower's, Lincoln, UK). Pots were placed into a ventilated polytunnel and stood on capillary

135   matting to allow for watering. Plants were grown on until they had begun to flower

136   (July/August) before being transferred to a ventilated glasshouse compartment (pansy plants)

137   or ventilated polytunnel (*Hebe* plants).

138

139   *Infesting plants with aphids*

140   A small number of pansy plants were placed into the insect cages in which *M. persicae* were

141   reared and a similar number of *Hebe* plants were placed into the insect cages in which *A.*

142   *gossypii* were reared. Plants were placed close together so that aphids were able to colonise

143   the pansy and *Hebe* plants. Once aphid populations had become established on pansy and

144   *Hebe* plants, completing at least two generations, these plants were then used to infest the

145   remaining pansy and *Hebe* plants. The remaining plants were infested by carefully placing

146   aphid infested leaves of the same plant species onto a previously uninfested plant. In this way

147   each plant was infested with approximately 10 mixed age aphids. Aphid populations were

148   allowed to establish for two weeks before the start of the experiment.

149

150   *Treatments*

151   Two conventional insecticides were used in this study, flonicamid (Mainman) and

152   spirotetramat (Movento) (see Table 1). Flonicamid works primarily through ingestion and

153   inhibits aphid feeding. Spirotetramat, also works mainly through ingestion, and is an acetyl-

154   CoA carboxylase inhibitor. Both insecticides are widely used for control of aphids on

155   ornamental crops. The three biopesticide products are based on plant essential oils (see Table

156   1). Azadirachtin A is obtained from neem seeds (*Azadirachta indica*) and is formulated as an

157   emulsifiable concentrate containing 1% azadirachtin A. *Chenopodium ambrosioides* variety

158   nr. *ambrosioides*, variously known as wormseed, Jesuit's tea or Mexican-tea is formulated as

159   an oil dispersion containing 16.75% of the essential oil, which is composed of a mixture of 14

160   monoterpenes. Orange oil is formulated as soluble liquid containing 60 g/l of the active

161   ingredient of which *d*-limonene is the major component.

162

163   *Treatment applications*

164 Treatments and a water control were applied using an Oxford Precision Sprayer (MDM

165 Engineering Ltd, Bristol, UK) fitted with a hollow cone nozzle (HC/1.74/3, Hypro EU Ltd,

166 Cambridge, UK) delivering a droplet size of 200-300 μm. Spray applications were made using

167 three 3 bar pressure. The concentration of each product used was recommended by the

168 manufacturer (see Table 1) and water volumes were standardised at 600 l/Ha.

169

170

171 *Experimental design*

172 Individual plots were created on benching within a glasshouse compartment or polytunnel

173 using panels of horticultural fleece fastened to a wooden frame to create open fronted cages.

174 Each side of the cage used a panel of horticultural fleece on each side of the frame in order

175 to create two layers of fleece between each adjacent cage. Plots were 0.5 m wide, 0.75 m

176 deep and 0.75 m high and plants were positioned as described in the centre of each plot and

177 so at least 10 cm away from the walls of the cage. The open top and front of each cage allowed

178 for ventilation and provided access for sampling and treatment application but importantly

179 reduced the movement of aphids between plots. Furthermore, as some components of the

180 biopesticide products tested are volatile the two layers of horticultural fleece minimised airflow

181 between plots. In order to prevent spray drift between plots or spray deposits coming into

182 contact with the wooden frame or horticultural fleece panels, solid plastic screens were placed

183 around the perimeter of each plot during spray application. Benches were lined with capillary

184 matting to allow for watering. A total of nine aphid infested plants, arranged in three rows of

185 three, were placed in each plot. Each of the following three experiments used a randomised

186 block design with each treatment replicated six times:

187

188 Experiment 1 – efficacy of biopesticides to control *M. persicae* on pansy plants under

189 glasshouse conditions. This experiment tested the relative efficacy of the three terpene based

190 biopesticide products compared with two synthetic insecticides as well as a water control (see

191 Table 1). In total, four applications of each treatment and the water control were applied;

192 applications were made at intervals of seven days.

193

194 Experiment 2 – as described for Experiment 1 but controlling *A. gossypii* on *Hebe* plants under

195 polytunnel conditions.

196

197 Experiment 3 – efficacy of biopesticides to control *A. gossypii* on *Hebe* plants under polytunnel

198 conditions. This experiment tested the relative efficacy of three terpene based biopesticide

199 products compared with the synthetic insecticide flonicamid as well as a water control. Unlike

200 in Experiment 2, where the number of applications and interval between each application was

201 standardised between each treatment, here the total number of applications and interval

202 between applications of each treatment over the 14 day experimental period was specified by

203 each manufacturer (see Table 1). As a result the number of applications ranged from between

204 one for flonicamid, made at the start of the experimental period, and five for Biopesticide 3

205 (orange oil) where applications were made at intervals of three days.

206

207 *Assessments*

208 Assessments of aphid numbers were done immediately before the first treatment application,

209 three and six days after the first treatment application and then weekly intervals over the

210 remainder of the experimental period. For each assessment of aphid numbers, all plants were

211 assessed, recording aphid numbers on the central/dominant stem of the plant. In each case

212 recording numbers of live aphids, of the species of interest, on the leaves, flowers and stems.

213 The presence of any other pests was also recorded along with the presence of any natural

214 enemies. Evidence of phytotoxicity caused by treatment application was also recorded each

215 time aphid numbers were recorded. Phytotoxicity assessments followed those developed by

216 the European and Mediterranean Plant Protection Organisation (guideline PP1/135). On each

217 assessment date all plants were assessed for evidence of inhibition of plant growth, thinning

218 of the plant, modification in plant colour (including both leaves and flower petals), evidence of

219 necrosis and deformations. Assessments were made by visually comparing the plants which

220 had been treated with a conventional synthetic insecticide or biopesticide product with control

221 plants. Finally, the temperature during each experiment was recorded using datalogger

222 (iButtons, HomeChip Ltd, Milton Keynes, UK).

223

224 *Statistical analyses*

225 All statistical analyses were performed using the statistics program Genstat 17th Edition

226 (Genstat, VSN International Ltd, Hemel Hempstead, UK). Each experiment was set out in a

227 randomised complete block design. Blocking was used to take into account any variability in

228 environmental conditions along the length of the polytunnel or glasshouse compartment with

229 increasing distance from the entrance. Weekly count data were analysed independently

230 using analysis of variance (ANOVA) with Ln (c=1) transformation of raw data. Individual

231 comparisons between treatments were made using Tukey's HSD test.

232

233

234 **Results**

235

236 Results and statistical analyses from Experiment 1 are summarised in Table 2. All products

237 tested had reduced numbers of *M. persicae* to zero, or close to zero, in each plot by the end

238 of the experimental period. Statistical analysis of the Ln (c=1) transformed aphid count data

239 shows that there was no significant difference in numbers of *M. persicae* between treatments

240 before the first spray application (mean aphid numbers per plot for each treatment and the

241 water control were between 20 and 32), but that there was a strong (P <0.001) treatment effect

242 for all assessments completed after this; i.e. from three days after the first the spray

243 application. No block effects were recorded on any of the assessment dates.

244

245 Individual comparisons between treatment means (indicated by Tukey's HSD test) showed

246 that numbers of *M. persicae* in plots treated with either Biopesticide 3 (active ingredient

247 orange oil) or the conventional insecticide Mainman (active ingredient flonicamid) were

248 significantly lower than in plots treated with the water control three days after the first spray

249 application. Of the other products tested, numbers of *M. persicae* in plots treated with

250 Movento (active ingredient spirotetramat), Biopesticide 1 (active ingredient azadirachtin A)

251 and Biopesticide 2 (active ingredient *Chenopodium ambrosioides* extract) were significantly

252 lower than in plots treated with the water control 6, 13 and 21 days after the first spray

253 application, respectively. There were no statistical differences between the numbers of

254 aphids recorded in plots treated with any of the conventional insecticides or biopesticides

255 tested from 21 days after the first spray application.

256

257 No effects on plant health (determined by assessments of plant growth, thinning of the plant,

258 evidence of necrosis and deformations) were recorded as a result of any treatment

259 application. Some slight colour changes to leaves and/or flowers were noted for all products

260 tested but were most apparent for Biopesticide products 1 (<5% of flowers and <1% of

261 leaves showing a slight colour change) and 3 (<5% of flowers and <1% of leaves similarly

262 showing a slight colour change) after each of the first three spray applications. There was no

263 evidence of any colour change to flowers or leaves after the fourth spray application. Each

264 product was applied when temperatures were between 16.5 and 18.6°C. There was no

265 evidence of any non-target effects of the products tested in this experiment, no other insects

266 were recorded on the plants throughout the assessment period.

267

268 Results and statistical analyses from Experiment 2 are summarised in Table 3. Of the

269 products tested, only the conventional insecticide Mainman had reduced numbers of *A.*

270 *gossypii* to close to zero in each plot by the end of the experimental period. The conventional

271 insecticide Movento and Biopesticide products 1, 2 and 3 had reduced aphid numbers by 80,

272 0, 44 and 0%, respectively, over the same period. Statistical analysis of the transformed

273 aphid count data showed that there was no difference in numbers of *A. gossypii* between

274 treatments and the water control before the first spray application (mean aphid numbers per

275   plot for each treatment and the water control were between 62 and 92). Statistical

276   differences were only apparent after plots had received three spray applications, i.e. 21 days

277   after the first spray application. No block effects were recorded on any of the assessment

278   dates.

279

280   Individual comparisons between treatment means showed that numbers of *A. gossypii* in

281   plots treated with the conventional insecticide Mainman were significantly lower than in plots

282   treated with the water control 21 days after the first spray application, by which time three

283   spray applications had been completed. Of the other products tested, numbers of *A. gossypii*

284   were significantly lower in plots treated with Movento compared with the water control. This

285   difference was, however, only recorded on the final assessment date, after four spray

286   applications had been applied. Numbers of *A. gossypii* in plots treated with Movento did not

287   differ from numbers of aphids recorded in plots treated with any of the biopesticide products.

288   None of the biopesticide products tested significantly reduced numbers of *A. gossypii*

289   relative to the water control.

290

291   No effects on plant health (determined by assessments of plant growth, thinning of the plant,

292   modification in plant colour (including both leaves and flower petals), evidence of necrosis

293   and deformations) were recorded as a result of any treatment application. Each product was

294   applied when temperatures were between 17.0 and 25.0°C. There was no evidence of any

295   non-target effects of the products tested in this experiment. Aphid parasitoids (*Aphidius*

296   spp.), seen as mummified aphids, were recorded at very low numbers (<1 per plant) in all

297   plots regardless of the treatment applied.

298

299   Results and statistical analyses from Experiment 3 are summarised in Table 4. Of the

300   products tested, all except Biopesticide 1 had reduced *A. gossypii* to low numbers in each

301   plot by the end of the experimental period. The conventional insecticide Mainman and

302   Biopesticide products 2 and 3 had reduced aphid numbers by 98, 81 and 90%, respectively,

303   over the same period. Statistical analysis of the transformed aphid count data showed that

304   there was no difference in numbers of *A. gossypii* between treatments and the water control

305   before the first spray application (mean aphid numbers per plot for each treatment and the

306   water control were between 28 and 32) and statistical differences were only apparent 14

307   days after the first spray application. No block effects were recorded on any of the

308   assessment dates.

309

310   Individual comparisons between treatment means shows that numbers of *A. gossypii* in plots

311   treated with either biopesticide products 2 or 3, or the conventional insecticide flonicamid,

312   were significantly lower than in plots treated with the water control 14 and 20 days after the

313   first spray application. Fourteen days after the first spray application plots had received one

314   application of Mainman, three applications of Biopesticide 2, and five applications of

315   Biopesticide 3. Twenty days after the first spray application numbers of *A. gossypii* in plots

316   treated with Mainman were significantly lower than in plots treated with Biopesticides 1 or 2

317   but were not statistically different from numbers in plots treated with Biopesticide 3.

318

319   No effects on plant health (determined by assessments of plant growth, thinning of the plant,

320   modification in plant colour (including both leaves and flower petals), evidence of necrosis

321   and deformations) were recorded as a result of any treatment application. Each product was

322   applied when temperatures were between 20.6 and 31.1°C. As for Experiment 2, there was

323   no evidence of any non-target effects of the products tested in this experiment. Aphid

324   parasitoids (*Aphidius* spp.), seen as mummified aphids, were recorded at very low numbers

325   (<1 per plant) in all plots regardless of the treatment applied.

326

327

328   **Discussion**

329

330 Biopesticides based upon plant extracts have a number of benefits when compared to their

331 conventional synthetic counterparts, including: little to no toxic residues, increased operator

332 safety, reduced development and production costs, multiple modes of action, and

333 compatibility with existing spray technologies (Hajek, 2004; Chandler et al., 2011; Pavela

334 and Benelli, 2016). However, the use of biopesticides based on plant extracts can also have

335 a number of disadvantages compared to conventional synthetic pesticides, including shorter

336 persistence and potential phytotoxic effects (Chandler et al., 2011). These disadvantages

337 typically mean that most biopesticides based on plant extracts are not as effective as

338 conventional synthetic pesticides and as a result are not suited for use as stand-alone

339 treatments, but may form an important part of an Integrated Pest Management (IPM)

340 programme.

341

342 This study tested the efficacy of three terpene based biopesticide products based on orange

343 oil, the essential oil from *Chenopodium ambrosioides* variety nr. *ambrosioides* or

344 azadirachtin A from neem oil, applied under commercial conditions against the aphid pests

345 *M. persicae* and *A. gossypii*. Results indicate that these products may be used both as part

346 of an IPM programme and, in some cases, may be used as a stand-alone treatment against

347 these pests. All three biopesticide products were notable for their overall efficacy against *M.*

348 *persicae* and, in the case of the orange oil based product, for its speed of kill against this

349 pest. The rapid speed of kill seen following application of the orange oil based product is

350 most likely due to its combined contact and possible fumigant action (Raina et al., 2007).

351 Indeed, statistically significant reductions in *M. persicae* populations on pansy plants were

352 seen just three days after application of this biopesticide product, similar to the results for the

353 conventional synthetic insecticide flonicamid and faster than the reductions observed for

354 spirotetramat. As both flonicamid and spirotetramat are widely used to control aphid pests of

355 ornamental crops the speed of kill reported here for orange oil, after a the first application, is

356 likely to be acceptable to growers wishing to control *M. persicae*.

357

358   The biopesticide products based on the *Chenopodium ambrosioides* extract or azadirachtin

359   A were slower acting, with statistically significant reductions in *M. persicae* populations on

360   pansy plants only seen 21 and 13 days after the first spray application, respectively.

361   Although the mode of action of the essential oil of *Chenopodium ambrosioides* is not known,

362   neem based biopesticides are known to be slower acting, displaying antifeedant and

363   repellent properties as well as inducing sterility in *M. persicae* (Mordue (Luntz) et al., 1996;

364   Chaudhary et al., 2017).

365

366   The overall efficacy against *M. persicae* of all three biopesticide products, after four spray

367   applications had been completed over a four week period, was not statistically different to

368   the conventional synthetic insecticides used. The biopesticide based on orange oil reduced

369   populations of *M. persicae* by 85%, the product based on the essential oil from

370   *Chenopodium ambrosioides* by 87% and the product based on azadirachtin A by 86%. By

371   comparison the conventional synthetic insecticides Mainman and Movento reduced aphid

372   numbers by 99 and 97%, respectively, in the same experiment. Results presented here

373   appear to be broadly similar to those reported by a Chiasson et al. (2004) who tested the

374   efficacy of a biopesticide product based on the essential oil from *Chenopodium*

375   *ambrosioides* against *M. persicae* on *Verbena*. This study used a similar methodology, but

376   found a biopesticide product based on this essential oil to be more effective against this pest

377   than a biopesticide based on azadirachtin A and similarly effective to an insecticidal soap

378   (potassium salts of fatty acids with 0.2% pyrethrins).

379

380   Speed of kill and overall efficacy was reduced when these biopesticides were applied

381   against *A. gossypii* on *Hebe*. This may indicate that the complex architecture of this crop

382   limited spray coverage. Good spray coverage is essential for biopesticides that display

383   contact activity (Copping and Menn, 2000). Evidence for the importance of spray coverage

384   can be seen most notably by the improved control of *A. gossypii* when the biopesticide

385   based on orange oil was applied to crops every three days in Experiment 3, as

386   recommended by the manufacturer, compared with every seven days, as in Experiment 2.

387   Indeed, when applied following label recommendations the biopesticide based on orange oil

388   gave a statistically similar level of control as the conventional synthetic insecticide Mainman.

389   The biopesticide based on the essential oil from *Chenopodium ambrosioides* was less

390   effective, but did significantly reduce populations of *A. gossypii* compared to the water

391   control. The biopesticide based on azadirachtin A was ineffective against this pest.

392

393   The requirement for good spray coverage and repeated applications of the biopesticide

394   products tested here to achieve effective control of crop pests highlights the need for

395   developments in biopesticide application techniques. For example, current standard practice

396   in the protected ornamentals industry is to use spray lance or pistol systems with extremely

397   high water volumes of 750-5,000 L/ha to apply pesticides to crops (Buxton, 2012). With such

398   high water volumes the time taken to apply pesticides is increased meaning that labour costs

399   increase. Furthermore, spray lance or pistol systems typically do not provide good spray

400   coverage of the underside of leaves (Derkson et al., 2008). Development and adoption of

401   more efficient and effective spray application techniques such as booms sprayer systems,

402   electrostatic sprayers, ultra-low volume (ULV) sprayers or gantry sprayer systems are likely

403   to improve both the efficacy and cost-effectiveness of biopesticides.

404

405   It has been noted that the essential oils that are most efficacious against pests are often the

406   most phytotoxic (Isman, 2000). While it is true that slight colour changes to petals and leaves

407   of pansy plants was most obvious following application of the biopesticide based on orange

408   oil, and to a lesser extent neem oil, the observed symptoms were rare and slight, with less

409   than 1% of leaves affected. Nonetheless, great care is required to avoid phytotoxic effects

410   when using biopesticides based on essential oils. Besides any phytotoxic effects resulting

411   from the use of plant essential oil based biopesticides it is also important to consider that

412   very little is known with regards to crop safety (Regnault-Roger et al., 2012), requiring further

413   work to comply with stringent legislation before commercialisation. Similarly, there is

414   comparatively little published information on the side-effects of plant essential oil based

415   biopesticides.

416

417   Results presented herein show that the three terpene based biopesticides have the potential

418   to reduce reliance on conventional synthetic pesticides by providing effective control of key

419   aphid pests of ornamental plants. This is particularly important as *M. persicae* and *A.*

420   *gossypii* have repeatedly developed resistance to conventional synthetic insecticides, such

421   as pyrethroids, carbamates and neonicotinoids (e.g. Furk and Hines, 1993; Bass et al.,

422   2011; Herron and Wilson, 2011; Foster and Blackshaw, 2012; Marshall et al., 2012, 2012).

423   Biopesticide products may, therefore, form an important component of an Insecticide

424   Resistance Management (IRM) programme to prevent or delay future cases of insecticide

425   resistance from developing. This may be achieved by alternating use of biopesticide

426   products with conventional synthetic insecticides and thereby rotating the modes of action

427   used to control these pests (Sparks and Nauen, 2015).

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523   Table 1. Products tested in this study. Minimum interval (days) between applications and

524   maximum number of applications permitted within experimental period columns apply only to

525   Experiment 3. For Experiments 1 and 2 a total of four applications of each treatment and the

526   water control were applied with each application made at intervals of seven days.

527

528   Table 2. Effect of treatments on *Myzus persicae*. Raw data transformed using Ln (c=1) and

529   presented as mean number of aphids/plot. Numbers in a column followed by the same letter

530   are not significantly different at P <0.05 based on individual contrasts (Tukey's HSD test).

531   DAIT = Days After Initial Treatment.

532

533   Table 3. Effect of treatments on *Aphis gossypii*. Raw data transformed using Ln (c=1) and

534   presented as mean number of aphids/plot. Numbers in a column followed by the same letter

535   are not significantly different at P <0.05 based on individual contrasts (Tukey's HSD test).

536   DAIT = Days After Initial Treatment.

537

538   Table 4. Effect of treatments on *Aphis gossypii*. Raw data transformed using Ln (c=1) and

539   presented as mean number of aphids/plot. Numbers in a column followed by the same letter

540   are not significantly different at P <0.05 based on individual contrasts (Tukey's HSD test).

541   DAIT = Days After Initial Treatment.

Product name	Active ingredient(s)	Active ingredient concentration	Formulation type	Application rate	Minimum interval (days) between applications	Maximum number of applications permitted within experimental period
Mainman	flonicamid	500 g/kg	wettable granule	0.14 kg/ha	21	1
Movento	spirotetramat	150 g/l	oil dispersion	0.5 l/ha	N/A	N/A
Biopesticide 1	azadirachtin A	1%	emulsifiable concentrate	0.5% (3.0 l/ha when applied in 600 l of water/ha)	7	2
Biopesticide 2	*Chenopodium ambrosioides* extract	16.75%	oil dispersion	0.67 v/v (4.0 l/ha if applied in 600 l water/ha)	5	3
Biopesticide 3	orange oil	60 g/l	soluble liquid	0.4% (2.4 l/ha when applied in 600 l of water/ha)	3	5

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Product name (active ingredient)	-1 DAIT	3 DAIT	6 DAIT	13 DAIT	21 DAIT	27 DAIT
Water control (n/a)	3.22^a	3.86^c	4.14^c	3.91^c	4.58^b	3.93^b
Movento (spirotetramat)	3.46^a	3.44^{bc}	2.12^{ab}	0.41^a	0.23^a	0.38^a
Mainman (flonicamid)	2.86^a	2.19^{ab}	0.88^a	0.30^a	0^a	0.12^a
Biopesticide 1 (azadirachtin A)	2.80^a	3.39^{bc}	2.85^{bc}	1.31^{ab}	0.83^a	1.31^a
Biopesticide 2 (*Chenopodium ambrosioides* extract)	2.91^a	3.65^c	3.15^{bc}	2.51^{bc}	1.03^a	0.98^a
Biopesticide 3 (orange oil)	2.82^a	1.49^a	1.13^a	1.12^{ab}	1.01^a	0.72^a
F value (5 d.f.)	1.14	10.29	14.26	12.44	19.78	15.90
Treatment	(P = n.s.)	(P <0.001)	(P <0.001)	(P <0.001)	(P <0.001)	(P <0.001)
F value (5 d.f.)	1.32	0.95	1.25	1.24	0.47	0.52
Block	(P = n.s.)	(P = n.s.)	(P = n.s.)	(P = n.s.)	(P = n.s.)	(P = n.s.)

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Product name/active ingredient	-1 DAIT	3 DAIT	6 DAIT	13 DAIT	21 DAIT	27 DAIT
Water control (n/a)	4.07[a]	3.83[a]	3.83[a]	4.26[a]	4.66[b]	4.89[c]
Movento (spirotetramat)	4.16[a]	3.63[a]	3.41[a]	2.97[a]	2.11[a,b]	2.40[a,b]
Mainman (flonicamid)	4.08[a]	3.52[a]	3.24[a]	2.23[a]	0.53[a]	0.35[a]
Biopesticide 1 (azadirachtin A)	4.39[a]	3.57[a]	3.50[a]	3.34[a]	3.14[a,b]	3.46[b,c]
Biopesticide 2 (*Chenopodium ambrosioides* extract)	4.20[a]	3.12[a]	3.23[a]	3.39[a]	3.29[b]	3.23[b,c]
Biopesticide 3 (orange oil)	4.12[a]	3.42[a]	3.49[a]	3.44[a]	3.33[b]	3.35[b,c]
F value (5 d.f.)	0.20	0.23	0.19	1.67	5.23	8.60
Treatment	(P = n.s.)	(P = n.s.)	(P = n.s.)	(P = n.s.)	(P = 0.002)	(P <0.001)
F value (5 d.f.)	0.69	0.68	0.48	0.87	0.73	1.74
Block	(P = n.s.)	(P = n.s.)	(P = n.s.)	(P = n.s.)	(P = n.s.)	(P = n.s.)

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Product name	-1 DAIT	3 DAIT	6 DAIT	14 DAIT	20 DAIT
Water control (n/a)	3.36^a	2.86^a	2.73^a	2.70^b	3.15^d
Mainman (flonicamid)	3.32^a	2.53^a	1.71^a	0.35^a	0.35^a
Biopesticide 1 (azadirachtin A)	3.39^a	2.65^a	2.50^a	2.80^b	2.66^{cd}
Biopesticide 2 (*Chenopodium ambrosioides* extract)	3.43^a	1.63^a	2.35^a	1.37^a	1.67^{bc}
Biopesticide 3 (orange oil)	3.26^a	2.41^a	1.84^a	1.10^a	1.27^{ab}
F value (4 d.f.)	0.14	2.27	2.14	11.36	13.24
Treatment	(P = n.s.)	(P = n.s.)	(P = n.s.)	(P <0.001)	(P <0.001)
F value (5 d.f.)	0.57	0.77	0.83	0.54	0.51
Block	(P = n.s.)	(P = n.s.)	(P = n.s.)	(P = n.s.)	(P = n.s.)

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