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"The Garlic Gambit": an alternative strategy for controlling vine weevil (*Otiorhynchus sulcatus* F.; Coleoptera: Curculionidae)

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Abstract

Plant protection products derived from plant material are proposed to be a sustainable alternative to conventional synthetic chemical pesticides. This study determines the efficacy of a commercially available bioinsecticide based on garlic (*Allium sativum* L.; Asparagales: Amaryllidaceae) extract against vine weevil (*Otiorhynchus sulcatus* F.; Coleoptera: Curculionidae) eggs and larvae in contact, fumigation and a combination of contact and fumigation bioassays under laboratory conditions. Results showed that garlic significantly reduced egg hatch rate compared to the control group when applied as fumigant. Similarly, egg hatch rate was reduced compared to the control group when garlic was applied as combined contact and fumigant applications. No effect was observed when the garlic product was applied as a contact application. The bioinsecticide significantly reduced larval survival when either contact or fumigant applications were used. A combined contact and fumigant effect was shown also when vine weevil eggs were exposed to the bioinsecticide for 30 days in plastic containers containing growing media. The number of larvae recovered after this period was significantly reduced compared to the control group.

This study demonstrates the potential of garlic-based bioinsecticides, such as Pitcher GR®, for vine weevil control. Further studies are, however, needed to determine the efficacy of such bioinsecticides under field conditions and investigate how these products can be most effectively used as a part of a wider vine weevil integrated pest management programme.

Keywords: Botanical bioinsecticide, contact test, fumigant test, egg hatchability, larval survival, weevil pest.

Introduction

Insect pest management has largely relied on synthetic chemical insecticides, such as carbamates, organophosphates, pyrethroids and neonicotinoids over the past six decades (Chandler *et al*. 2011). Whilst these products have proven to be effective, alternative and more sustainable control measures are required to avoid environmental contamination, reduce risk to humans and beneficial organisms and to combat the increasing number of cases of insect resistance in pest populations (Barzman *et al*. 2015, Chaudhary *et al*. 2017). One such alternative is the use of botanical bioinsecticides formulated using naturally occurring secondary metabolites (e.g., fatty acids, alkaloids, terpenoids, phenols, quinones, tannins and flavonoids) extracted from plant material (roots, leaves, stems, fruits, etc.) (Pavela 2016). These phytochemicals have been shown to be effective for controlling insect pests by disrupting key physiological processes, such as moulting and respiration as well as exciting the central nervous system (Pavela and Benelli 2016, Lengai *et al*. 2020). Phytochemicals have also been shown to modify insect behaviour by acting as attractants, deterrents, phago-stimulants, antifeedants or anti-oviposition cues (Isman 2006). The bioactivity of many phytochemicals on a wide range of insect pests is complemented by their low cost, lack of residual effects, availability and compatibility with circular economy principles (Isman 2006). Due to their chemical complexity, phytochemicals are also less susceptible to pest populations developing resistance as there may be several modes of action (Lengai *et al*. 2020). Botanical bioinsecticides, therefore, represent a useful tool that could be incorporated into integrated pest management (IPM) programmes alongside conventional synthetic insecticides (Isman *et al*. 2011) and biological control agents (Benelli *et al*. 2018).

Garlic (*Allium sativum* L.; Asparagales: Amaryllidaceae) is a perennial species of bulbous flowering plant closely related to onions, leeks and chives native to Asia (Block 2009).

This plant is widely cultivated for culinary use as a seasoning due to its distinct flavour profile resulting from high levels of bioactive phytochemicals found in the bulb, including allicin (2-propene-1-sulfinothioic acid *S*-2-propenyl ester), diallyl sulfide (3-prop-2-enylsulfanylprop-1-ene), diallyl disulfide (3-(prop-2-enyldisulfanyl)prop-1-ene) and diallyl trisulfide (3-(prop-2-enyltrisulfanyl)prop-1-ene) (Huang *et al*. 2000).

Garlic extracts (e.g., oil and powder) have been shown to possess insecticidal, repellent, anti-feeding and anti-microbial properties (Mamduh *et al*. 2017). Furthermore, garlic extracts applied as fumigant and contact applications have been reported to induce lethal and sublethal effects to several stored product pest species, including the rice weevil (*Sitophilus oryzae* L.; Coleoptera: Curculionidae), the maize weevil (*S. zeamais* Motschulsky; Coleoptera: Curculionidae) and the red flour beetle (*Tribolium castaneum* Herbst; Coleoptera: Tenebrionidae) (Ho *et al*. 1996, Huang *et al*. 2000). In contact bioassays, for example, garlic essential oil has been reported to induce symptoms such as necrosis in mealworm beetle (*Tenebrio molitor* L.; Coleoptera: Tenebrionidae) larvae, pupae and adults twenty-forty hours after exposure (Plata-Rueda *et al*. 2017). Other effects of garlic extracts include decreased hatching rates and embryonic development (Mamduh *et al*. 2017). Diallyl trisulfide, an active substance from garlic essential oil, has been shown to significantly downregulate the mRNA *of* chitin synthase A (CHSA) gene (a conserved enzyme responsible for the completion of chitin biosynthetic pathway) in the Angoumois grain moth (*Sitotroga cerealella* Olivier; Lepidoptera: Gelechiidae) (Shah *et al*. 2020). Downregulation of CHSA resulted in chitin reduction, epidermal disruption, morphological and physiological alterations as well as oviposition reduction (Shah *et al*. 2020). Currently, there are several products based on garlic extracts and these are marketed (e.g., in the United States and Canada) as bioinsecticides and repellents for the management of many insect pests (Dusi *et al*. 2022).

Vine weevil (*Otiorhynchus sulcatus* F.; Coleoptera: Curculionidae) is one of the most economically important horticultural insect pests worldwide (Pope and Roberts 2022). Larvae feed on subterranean plant tissues while adults feed on plant foliage and form characteristic notching along the leaf margin. Feeding damage by larvae negatively impacts plant health and yield while adult feeding significantly reduces the economic value of ornamental plants where aesthetic appearances are important (Pope and Roberts 2022). Current approaches to vine weevil management rely on entomopathogenic fungi (EPFs) (Pope and Roberts 2022) and in particular on entomopathogenic nematodes (EPNs), using species belonging to the Heterorhabditis and Steinernema genera (Lola-Luz and Downes 2007, Haukeland and Lola-Luz 2010). However, the use of EPNs for vine weevil control should be considered as one component of an IPM programme rather than as a direct replacement for conventional synthetic insecticides. Therefore, development of novel tools and the integration of these tools into IPM programmes needs to be achieved.

This study evaluated the potential role of a granular garlic-extract bioinsecticide, Pitcher GR®, commercially available in UK as a management option for vine weevil eggs and larvae under laboratory conditions.

Materials and Methods

Insect Cultures

Adult vine weevils were collected from commercial strawberry (*Fragaria* × *ananassa cv. Duchesne*) crops in Staffordshire (UK) during summer 2022. An equal number of adults (e.g., thirty) was placed into each terrarium (*N* = 4; 30 × 19.3 × 20.6 cm; Exo Terra, Castleford, UK) containing yew (*Taxus baccata* L.; Cupressales: Taxaceae) sprigs with moist paper towels as a food/water source and cotton wool balls to act as an oviposition site. Yew, paper towels and cotton wool balls were replaced weekly. These terraria were housed in a controlled environment room maintained at 20 °C and 60 % relative humidity with a 16:8 light:dark photoperiod (Fitotron, Weiss Technik, Ebbw Vale, Wales, UK).

Bioinsecticide Treatment

Pitcher GR® (ICL Specialty Fertilizers, UK) is a granular bioinsecticide based on garlic extract (active ingredient: 450 g / kg garlic extract) commercially available in the United Kingdom (approved through an Extension of Authorisation for Minor Use; Ministerially Approved Pesticide Product (MAPP) Number: 18126) for the control of vine weevil eggs and young larvae in containerised ornamental plants (ICL Specialty Fertilizers 2024). The recommended application rate is 240 kg per ha (24 g per m^2). Based on this, 0.42 g or 0.06 g used in the laboratory experiments were calculated for the area of the plastic containers (170 cm^2) and Petri dishes (28.26 cm^2), respectively.

Toxicity Screening on Vine Weevil Eggs

Egg Collection

Eggs were collected every three days from the cotton wool balls in each terrarium using a fine 000 paintbrush. All eggs were examined under a binocular microscope (Microtec HM3, Somerset, UK) to ensure that only undamaged, fully melanised eggs were used for an experimental batch. These experimental batches (approximately 100 eggs) were then stored at 5 °C (Lec LSR151, Merseyside, UK) in plastic containers (115 x 40 mm, Signature Packaging, UK) until they were used in a bioassay. Eggs were used in bioassays within seven days of collection from the terraria. Regular egg viability counts were carried out by maintaining twenty eggs on moist tissue paper in a 90 mm diameter plastic Petri dish (Thermo Scientific™ 101VR20, Fisher Scientific UK Ltd) in a controlled environment room (Fitotron) at 20 °C and 60 % relative humidity in complete darkness. The remaining egg batches were maintained under the same environmental conditions in plastic containers (17 x 10.2 x 3 cm, GP Globe Packaging, UK) filled with compost (SylvaGrow® John Innes No. 2, Tetbury, UK) within a controlled environment room under the same conditions previously described (Fitotron) to produce larvae for toxicity screening.

Bioassay Experimental Units

Laboratory bioassays were carried out to determine the toxicity of Pitcher GR® through contact and fumigation as well as combined contact and fumigation on vine weevil eggs. Experimental units consisted of plastic containers (17 x 10.2 x 3 cm, GP Globe Packaging, UK) (Fig. 1 a) lined with two filter papers (Ø = 110 mm; Fisherbrand™, Fisher Scientific UK Ltd). Thirty vine weevil eggs were then transferred to each container using a fine 000 paintbrush. Experimental units were then placed in a controlled environment room maintained at 20 °C and 60 % relative humidity (Fitotron) under complete darkness. Eggs were monitored daily for thirty days and at the end of this period, egg viability was assessed by counting the total number of hatched eggs. Eggs were considered dead when they became opaque, necrotic and/or appeared dehydrated.

Contact Toxicity Bioassay

Contact toxicity of Pitcher GR® to vine weevil eggs was determined in a direct contact bioassay. This was achieved by using the experimental units previously described in the Bioassay Experimental Units Section. In the control experimental units 3 ml of water was pipetted into filter papers while 0.42 g of Pitcher GR® and 3 ml of water was added to the filter papers in the treatment experimental units. Adding water to the experimental units ensured that the eggs were hydrated and the filter papers were re-wetted with 1 ml of water as required. Experimental units were left unsealed in this bioassay to minimise the potential fumigant effect of Pitcher GR®. Ten replicate experimental units were prepared for each control and treatment with a total of 600 eggs tested (300 in the treated group and 300 in the untreated group).

Fumigant Toxicity Bioassay

Fumigant toxicity of Pitcher GR® to vine weevil eggs was determined in a non-contact fumigation bioassay. This was achieved by using the experimental units previously described for the Contact Toxicity Bioassay but adding Petri dishes (35 x 11 mm; Fisher Scientific, UK Ltd) containing either 3 ml of water (control experimental units) or 3 ml of water and 0.42 g of Pitcher GR® granules (treatment experimental units) to prevent direct contact between the vine weevil eggs and Pitcher GR® granules. Experimental units were sealed with airtight lids. All the experimental units initially received 3 ml of water to their filter papers to ensure that eggs were sufficiently hydrated and re-wetted with 1 ml of water as required. Ten replicate experimental units were prepared for each control and treatment with a total of 600 eggs tested (300 in the treated group and 300 in the untreated group).

Combined Contact and Fumigant Toxicity Bioassay

Combined contact and fumigant toxicity of Pitcher GR® to vine weevil eggs was determined in a contact fumigation bioassay. This was achieved using the bioassay set up described for the Contact Toxicity Bioassay but also sealing the experimental units with airtight lids. In the control experimental units 3 ml of water was pipetted onto filter papers while 0.42 g of Pitcher GR® and 3 ml of water was added to the filter papers in the treatment experimental units. All experimental units initially received 3 ml of water to their filter papers to ensure that eggs were sufficiently hydrated and re-wetted with 1 ml of water as required. Ten replicate experimental units were prepared for each control and treatment with a total of 600 eggs tested (300 in the treated group and 300 in the untreated group).

Toxicity Screening on Vine Weevil Larvae

Larva production

Vine weevil eggs collected as described in the Egg Collection Section were maintained in plastic containers (17 x 10.2 x 3 cm, GP Globe Packaging, UK) filled with growing media (SylvaGrow® John Innes No.2). Carrot slices were placed on the surface of the growing media each week in plastic containers as a food source for emerging larvae. These containers were housed in a controlled environment room at 20 °C and 60 % relative humidity (Fitotron) under complete darkness to produce larvae for toxicity screening.

Bioassay Experimental Units

Laboratory bioassays were carried out to determine the toxicity of Pitcher GR® through contact or fumigation as well as combined contact and fumigation on vine weevil larvae. Experimental units consisted of plastic Petri dishes (60 x 15 mm; Sarstedt AG & Co. KG, Germany) (Fig. 1 b) lined with filter papers (Ø = 47 mm; Whatman™, Fisher Scientific UK Ltd). Individual vine weevil larvae (first-instar larvae, max 20 days old; body length ≈ 3.2 mm, head capsule width ≈ 0.62 mm) were added to each Petri using a 000 fine paintbrush. Once the treatments being tested were added to these experimental units, they were maintained under complete darkness in a controlled environment cabinet (Panasonic, MLR- 352- PE, Japan) set to 20 °C. A carrot slice (Ø = 1 cm) was placed in each experimental unit as a food source. Larvae were monitored daily for seven days and after this period the number of surviving individuals was recorded. Larval mortality was determined by gently stimulating individuals with a 000 fine paintbrush and observing movement, with no movement indicating mortality.

Contact Toxicity Bioassay

Contact toxicity of Pitcher GR® to vine weevil larva was determined in a direct contact bioassay. This was achieved by using the experimental units previously described in the Bioassay Experimental Units Section. In the control experimental units 2 ml of water was pipetted into filter papers while 0.06 g of Pitcher GR® and 2 ml of water was added to the filter papers in the treatment experimental units. Adding water to the experimental units ensured that larvae were hydrated, and the filter papers were re-wetted with 1 ml of water as required throughout the bioassay. Experimental units were left unsealed in this bioassay to eliminate the potential fumigant effect of Pitcher GR®. Thirty replicate experimental units were prepared for each control and treatment with a total of 60 larvae tested (30 in the control group and 30 in the treatment group).

Fumigant Toxicity Bioassay

Fumigant toxicity of Pitcher GR® to vine weevil larvae was determined in a non-contact fumigation bioassay. This was achieved by using the experimental units previously described for the Contact Toxicity Bioassay but attaching with glue a smaller Petri dish (35 x 10 mm; Sarstedt AG & Co. KG, Germany) containing 2 ml of water (control experimental units) or 2 ml of water and 0.06 g of Pitcher GR® granules (treatment experimental units) to the larger Petri dish. All the experimental units were sealed with airtight lids. A drill (Dremel 3000, Eternal Tools, UK) was used to make a 1 cm hole in the larger Petri dishes to allow volatiles from the treatment to enter the experimental unit. Fine mesh was glued over this hole to prevent direct contact between the larvae and the treatments. Adding water to the experimental units ensured that larvae were hydrated, and the filter papers were re-wetted with 1 ml of water as required throughout the bioassay. Thirty replicate experimental units were prepared for each control and treatment with a total of 60 larvae tested (30 in the control group and 30 in the treatment group).

Combined Contact and Fumigant Toxicity Bioassay

Combined contact and fumigant toxicity of Pitcher GR® to vine weevil larvae was determined in a contact fumigant bioassay. This was achieved using the bioassay set up described for the Contact Toxicity Bioassay but also sealing the experimental units with airtight lids. In the control experimental units 2 ml of water was pipetted into filter papers while 0.06 g of Pitcher GR® and 2 ml of water was added to the filter papers in the treatment experimental units. Adding water to the experimental units ensured that larvae were hydrated, and the filter papers were re-wetted with 1 ml of water as required throughout the bioassay. Thirty replicate experimental units were prepared for each control and treatment with a total of 60 larvae tested (30 in the control group and 30 in the treatment group).

Growing Media Bioassay

Combined contact and fumigant toxicity of Pitcher GR® to vine weevil eggs under semi-field conditions was determined in a contact and fumigant bioassay. This was achieved by taking the experimental units (e.g., plastic containers) described in the Bioassay Experimental Units Section and filling with 100 g of growing media (SylvaGrow® John Innes No.2) before adding thirty vine weevil eggs onto the growing media surface using a 000 fine paintbrush then sealing with airtight lids. In the control experimental units 3 ml of water was added while 0.42 g of Pitcher GR® and 3 ml of water was added in the treatment experimental units. All experimental units initially received 3 ml of water to ensure that eggs were sufficiently hydrated and re-wetted with 1 ml of water as required. Carrot slices were placed in each experimental unit as a food source for emerging larvae. Experimental units were assessed after 30 days with the number of larvae recorded per unit. Ten replicate experimental units were prepared for each control and treatment with a total of 600 eggs tested (300 in the treated group and 300 in the untreated group).

Statistical Analysis

Statistical analyses were carried out using R v 4.3.2 (R Core Team 2022). In bioassays testing the toxicity of Pitcher GR® to vine weevil eggs through different exposures routes, count data were analysed using generalised linear models (GLMs) fitted to a Poisson or quasi-Poisson probability distribution depending on the presence of overdispersion. This was determined using *qcc.overdispersion.test* function from the *qcc* package (Scrucca 2004), where a *p*-value greater than 0.05 indicated no overdispersion and justifies the use of Poisson distribution. Count data for larvae were analysed with GLMs fitted to a binomial probability distribution as there were only two outcomes in this bioassay: live or dead. To analyse the mean of larvae recovered in the Growing Media Bioassay, count data were analysed using GLMs fitted to a Poisson probability distribution after testing for overdispersion as previously described.

Results

Vine Weevil Eggs

Contact Toxicity Bioassay

Exposing vine weevil eggs to Pitcher GR® in a direct contact toxicity bioassay had no effect on egg hatch rate compared to those exposed to water (GLM with a Poisson distribution: $z = -1.069$, df = 18, $p = 0.285$). The mean percentage of hatched eggs for the control treatments was 95 ± 1.3 %, while the Pitcher GR® treatment exhibited a mean hatch rate of 87.0 ± 2.3 % (Fig. 2a).

Fumigant Toxicity Bioassay

Exposing vine weevil eggs to Pitcher GR® in a fumigation toxicity bioassay significantly reduced egg hatch rate compared to those exposed to water (GLM with a quasi-Poisson distribution: $t = -12.32$, df = 18, $p < 0.001$). The mean percentage of hatched eggs decreased from 91.7 ± 1.1 % in the control treatment to 4.0 ± 1.4 % in the Pitcher GR® treatment (Fig. 2b).

Combined Contact and Fumigant Toxicity Bioassay

Exposing vine weevil eggs to Pitcher GR® in a contact fumigation toxicity bioassay significantly reduced egg hatch rate compared to those exposed to water (GLM with a quasi-Poisson distribution: $t = -5.678$, $df = 18$, $p < 0.001$). The mean percentage of hatched eggs decreased from 97.3 ± 1.2 % in the control treatment to 1.7 ± 1.7 % in the Pitcher GR® treatment (Fig. 2c).

Vine Weevil Larvae

Contact Toxicity Bioassay

Exposing vine weevil larvae to Pitcher GR® in a direct contact toxicity bioassay significantly increased larval mortality compared to those exposed to water (GLM with a binomial distribution: $z = -3.239$, $df = 58$, $p = 0.0012$). The mean percentage of live larvae for the control treatment was 96.7 ± 3.3 %, while the Pitcher GR® treatment exhibited a mean survival rate of 46.7 ± 8.9 % (Fig. 3a).

Fumigant Toxicity Bioassay

Exposing vine weevil larvae to Pitcher GR® in a fumigation toxicity bioassay significantly increased larval mortality compared to those exposed to water (GLM with a binomial distribution: $z = -4.066$, $df = 58$, $p < 0.001$). The mean percentage of live larvae decreased from 70.0 ± 9.2 % in the control treatment to 13.3 ± 5.4 % in the Pitcher GR® treatment (Fig. 3b).

Combined Contact and Fumigant Toxicity Bioassay

Exposing vine weevil larvae to Pitcher GR® in a contact fumigation toxicity bioassay significantly increased larval mortality compared to those exposed to water (GLM with a binomial distribution: z = -5.081, df = 58, $p < 0.001$). The mean percentage of live larvae decreased from 93.3 ± 4.4 % in the control treatment to 10.0 ± 5.1 % in the Pitcher GR® treatment (Fig. 3c).

Combined Contact and fumigant test with growing media

Exposing vine weevil eggs to Pitcher GR® in a contact fumigation toxicity bioassay under semi-field conditions significantly reduced larval eclosion rate compared to those exposed to water (GLM with a quasi-Poisson distribution: t = -7.415, df = 18, $p < 0.001$). The mean percentage of larval eclosion decreased from 62.7 ± 5.9 % in the control treatment to 19.7 ± 1.9 % in the Pitcher GR® treatment (Fig. 4).

Discussion

Concerns regarding the potential risks that synthetic chemical insecticides pose to human and environmental health have led to the development of alternative approaches to managing insect pests in agricultural production systems. Natural products such as phytochemicals derived from plants offer a potential solution to address this need and it is widely believed that they can play an important role in sustainable agriculture (Campos *et al*. 2019, Lengai *et al*. 2020). Such products have been shown to be effective against several economically important weevil (Curculionidae) pests. For example, neem seed extract, containing 4.5 % azadirachtin, has been reported to reduce the survival of the root weevil (*Diaprepes abbreviates* L.) larvae in laboratory and greenhouse experiments (Weathersbee and Tang 2002). Parwada *et al.* (2018) reported the lethal effect of three botanical pesticides, the forest red gum (*Eucalyptus terreticonis* Sm; Myrtales: Myrtaceae), the mint marigold (*Tagetes minuta* L.; Asterales: Asteraceae) and the common lantana (*Lantana camara* L.; Lamiales: Verbenaceae) against the maize weevil (*Sitophilus zeamais* Motschulsky).

Powders, extracts, essential oils and purified compounds from garlic have been reported to have lethal and sub-lethal effects to a number of pest species from the Coleoptera, Lepidoptera, Hemiptera and Diptera insect orders (Huang *et al*. 2000, Yang *et al*. 2012, Ramzi *et al*. 2022). Acetone leaf extracts of garlic have been shown, for example, to reduce survival of all red flour beetle (*Tribolium castaneum* Herbst; Coleoptera: Tenebrionidae) life-cycle stages under laboratory conditions (Ali *et al*. 2014). Similarly, volatile chemicals associated with garlic were recorded to be effective against adults of the cowpea weevil (*Callosobruchus maculatus* Fabricius; Bruchidae: Coleoptera) at all the concentrations tested between 0.1 % and 20 % (Abdalla *et al*. 2017). Under glasshouse conditions, a garlic extract product (Ecoguard- granules; Ecospray Limited) at a dose equivalent to the rate (0.12 g product/plant) has been shown to reduce by almost 60 % the numbers of cabbage root fly (*Delia radicum* L.; Diptera: Anthomyiidae) larvae/pupae (Jukes *et al*. 2005).

In line with these previous studies, this study demonstrated that the garlic-based bioinsecticide Pitcher GR® is effective against vine weevil eggs and larvae under laboratory conditions. In particular, this garlic-based product reduced egg hatch when applied as a fumigant and when contact and fumigant applications were combined. No significant effect of this product was observed when the product was applied with contact activity only. The reduction in hatch rate of eggs due to the fumigant and contact activity of garlic-based products have been previously documented (Ekesi 2000). For example, Ho *et al.* (1996) showed that oil from fresh garlic cloves caused mortality in red flour beetle eggs and that eggs were the most susceptible stage compared to adults, ten day old larvae and older larvae. In another study, two of the major constituents of garlic oil, namely methyl allyl disulfide and diallyl trisulfide, have been also reported to reduce egg hatching of the red flour beetle (Huang *et al*. 2000). Prowse *et al*. (2006) reported that a commercial garlic juice-based insecticide was effective against the cabbage root fly (*Delia radicum* L.; Diptera: Anthomyiidae) and the housefly (*Musca domestica* L.; Diptera: Muscidae) eggs. However, it was not fully understood if the reduction in hatch rate was due to contact or fumigant toxicity.

Garlic extracts are believed to contribute to increase insect mortality by preventing larval eclosure (Jarial 2001). Some garlic bioactive components are thought to cause lethal physiological changes during the embryonic development, so that hatching is inhibited (Prowse *et al*. 2006). These findings are promising and suggest the potential of garlic-based products as possible strategy for pest control.

In the present study, survival of vine weevil larvae was reduced when the garlic-based product was applied in a way that contact, fumigant or both contact and fumigant effects were possible. This study did not investigate the underlying modes of action, however, previous studies have reported that some enzymes such acetylcholinesterase can be inhibited by garlic extracts (Bhatnagar-Thomas and Pal 1974, Singh and Singh 1996). In addition, garlic constituents have been shown to affect the respiration rate, leading to physiological stress and paralysis of insects (Plata-Rueda *et al*. 2017). Allicin, the major component present in garlic, has been reported to cause embryonic death as well as affect fertility, fecundity and the production process of moulting hormones (Mamduh *et al*. 2017). Despite this, the modes of action of garlic extracts are still not fully understood and further studies are, therefore, needed.

The efficacy of garlic and its constituents in controlling insect pests has been shown to be affected by a variety of biological factors, including pest development stage. For example, Plata-Rueda *et al*. (2017) showed that the mealworm beetle (*Tenebrio molitor* L.; Coleoptera: Tenebrionidae) was more susceptible to two garlic compounds, diallyl sulfide and diallyl disulfide, in the pupal stage, with the larval and adult stages less susceptible. The authors suggested that efficacy may be influenced by the penetration of the garlic compounds into the body of the insect and the ability of the insect to metabolize these compounds. However, further investigation into how garlic affects insect pests at different developmental stages is needed.

The susceptibility of eggs and young larvae to garlic recorded in this study is likely to have a bearing on how this product can be used in the field. It is believed that these stages are most susceptible to botanical bioinsecticides (Erler *et al*. 2010, Kedia *et al*. 2015). If true, this would mean that like other biopesticides, application timing would be important to effectively target these stages of vine weevil life cycle (Pope and Roberts 2022). In addition, the method of application is also likely to be crucial in targeting these stages as this will determine whether contact or fumigant activity is achieved. Additional research is also necessary to investigate the effect of garlic on other vine weevil life stages, such as late instar larvae, pupae and adults.

At the same time there is, to date, a relative lack of knowledge on the efficacy of botanical bioinsecticides against below-ground insect pests such as weevils. This is surprising because some botanical bioinsecticides, such as neem (*Azadirachta indica* A. Juss; Sapindales: Meliaceae), have been used as soil amendments against plant parasitic nematodes (Abbasi *et al*. 2005) and fungal pathogens (Khan *et al*. 2020). However, only few botanical bioinsecticides are available for soil application against below-ground insect pests. Further studies are, therefore, required to improve the use of such products, including Pitcher GR®, against weevil pests and promote their incorporation into IPM programmes.

Although garlic-based oils and extract formulations have been studied in many laboratory tests, relatively few studies present results from its practical use. Plant extracts efficacy in the field is variable and extremely contingent on prevailing environmental and weather conditions (Lengai *et al*. 2020). These include temperature, relative humidity, level of sunlight radiation, altitude, photoperiod and type of soil as well as ecological interactions (e.g., herbivory or mutualism) (Souto *et al*. 2021). Consequently, variables influencing the efficacy of plant extracts need to be investigated under commercial field conditions, in order to better understand the potential of their use as alternatives to synthetic chemical pesticides.

Recent studies have highlighted that using two different management strategies together can result in higher pest suppression than when used individually (Reddy and Chowdary 2021). Botanical bioinsecticides are often used in conjunction with other IPM strategies such as entomopathogenic nematodes (EPNs) or natural enemies (Amoabeng *et al*. 2019, Reddy and Chowdary 2021). This reduces the cost of biological control applications as well as grower reliance on synthetic chemical insecticides (Guo *et al*. 2016). Initial research has shown that plant-based products may not affect the survival, virulence or infectivity of

EPNs and natural enemies when combined. For example, Laznic and Trdan (2014) reported the compatibility of azadirachtin with *Steinernema feltiae* (Filipjev; Rhabditida: Steinernematidae) and *Heterorhabditis bacteriophora* (Poinar; Rhabditida: Heterorhabditidae). Therefore, it would be beneficial to also assess the compatibility of Pitcher GR® with other vine weevil control strategies. Compatibility between different IPM strategies may promote the combined use of EPNs and botanical bioinsecticides, including Pitcher GR®, as a cost-effective alternative strategy for vine weevil management.

Insects have been reported to develop adaptations to toxic secondary plant metabolites (allelochemicals) in a number of plant systems, which has the potential to reduce the efficacy of allelochemicals used in pest management programmes (War *et al*. 2020). The adaptations to plant secondary metabolites can happen either by degradation, detoxification, sequestration or excretion. For example, the tobacco aphid (*Myzus nicotianae* Blackman; Hemiptera: Aphididae) has evolved the ability to detoxify nicotine and so enhance tolerance to the pyridine alkaloid nicotine (Bass *et al*. 2013), a group of toxic compounds produced by tobacco (*Nicotiana attenuate* Torr. ex S.Watson; Solanales: Solanaceae) and other members of the Solanaceae family. Therefore, there is a need to understand the underlying mechanism of insect adaptation to plant secondary metabolites as well as identify genes that are involved in the insect resistance to plant toxins. Little is known about the ability of vine weevil to adapt to allelochemicals, however, the availability of an annotated genome and knowledge of how this species interacts with host plants would enable informed decisions to be made on the use of phytochemical based products.

This study demonstrates the potential of garlic-based bioinsecticides such as Pitcher GR® to suppress vine weevil egg and larval development in controlled conditions. The product's multiple toxic effects (contact, fumigant and both combined toxicity) are particularly promising for effective control. While field trials are essential to validate efficacy in outdoor settings, these results establish a strong foundation for developing garlic-based bioinsecticides as a sustainable vine weevil management tool. Further research should focus on optimizing application strategies in the field and exploring synergies between Pitcher GR® and other IPM tactics.

Author contribution statement

Eugenia Fezza: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Data curation, Writing – original draft, Writing - review & editing, Visualisation. **Joe M. Roberts**: Conceptualization, Methodology, Data curation, Writing – original draft, Writing - review & editing, Visualisation, Supervision. **Toby J. A. Bruce:** Writing - review & editing. **Lael E. Walsh:** Writing - review & editing. **Michael T. Gaffney:** Conceptualization, Methodology, Writing – original draft, Writing - review & editing, Supervision. **Tom W. Pope:** Conceptualization, Methodology, Data curation, Writing – original draft, Writing - review & editing, Supervision.

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Declaration of competing interest

The authors declare that they have no conflicts of interest. The funders had no role: in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

Data availability

Data will be made available on request.

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Figure captions

Figure 1. Schematic diagram showing the experimental set-up used to determine the toxic effect of a garlic-based product on vine weevil eggs (a) and larvae (b).

Figure 2. Percentage (mean ± SE) of hatched eggs recorded when testing the effect of a garlic-based product (Pitcher GR®) in contact (a), fumigant (b) and combined contact and fumigant (c) tests. Thirty eggs (number of replicates = 10) were used for testing the effect of Pitcher GR® on vine weevil. Asterisks indicate significant differences between treated (Pitcher GR®) and untreated group (Control) (GLMs with a quasi-Poisson or Poisson distribution: *** = $p < 0.001$; NS = not significant).

Figure 3. Percentage (mean ± SE) of live larvae recorded when testing the effect of a garlic-based product (Pitcher GR®) in contact (a), fumigant (b) and combined contact and fumigant (c) tests. A single larva (number of replicates = 30) was used for testing the effect of Pitcher GR® on vine weevil. Asterisks indicate significant differences between treated (Pitcher GR®) and untreated group (Control) (GLM with a binomial distribution: ** = $p < 0.05$; *** = $p < 0.001$).

Figure 4. Percentage (mean ± SE) of larvae eclosion recorded when testing the effect of a garlic-based product (Pitcher GR®) in combined contact and fumigant test with growing media. Thirty eggs (number of replicates = 10) were used for testing the effect of Pitcher GR® on vine weevil. Asterisks indicate significant differences between treated (Pitcher GR®) and untreated group (Control) (GLM with a quasi-Poisson distribution: *** = $p < 0.001$).

Graphical Abstract Figure. "The Garlic Gambit": an alternative strategy for controlling vine weevil (*Otiorhynchus sulcatus* F.; Coleoptera: Curculionidae) .

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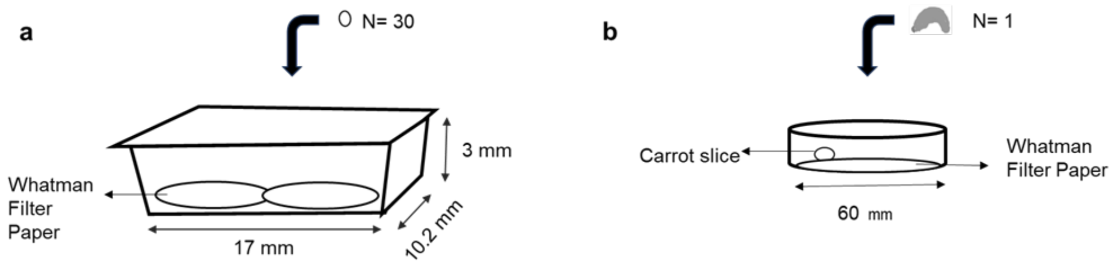
The garlic-based bioinsecticide resulted to be effective against vine weevil. In fact, the tested product reduced significantly vine weevil hatch rate in fumigant and combined contact and fumigant applications. Similarly, vine weevil larvae were also negatively affected by garlic in all the types of applications. Furthermore, the number of larvae recorded in combined contact and fumigant test with growing media was significantly reduced compared to the control group.


a
N= 30
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Paper
17 mm
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b
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Whatman
Filter Paper
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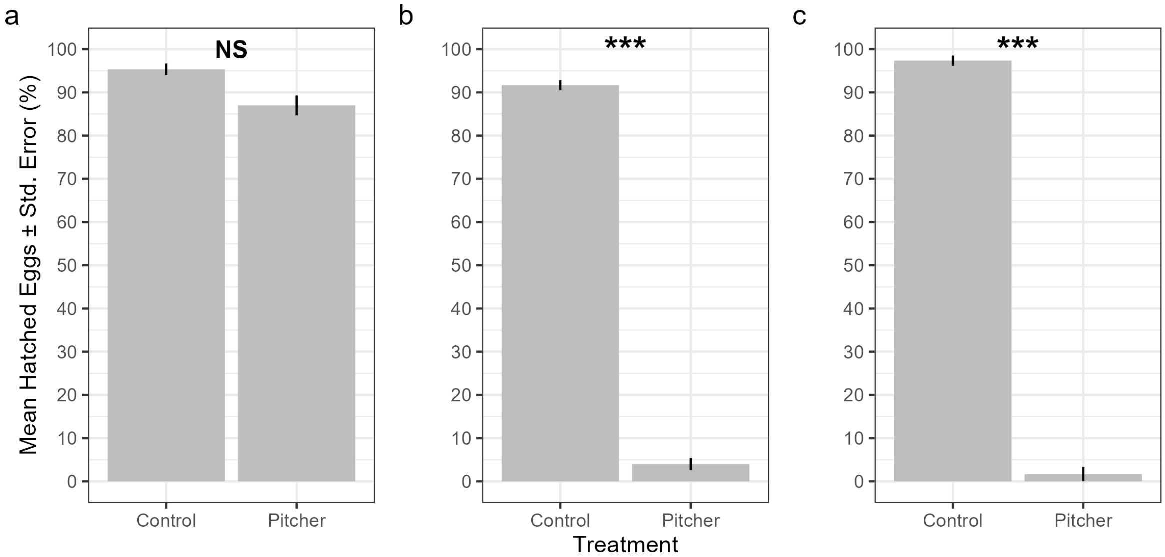
Figure 1


a
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c
NS
***
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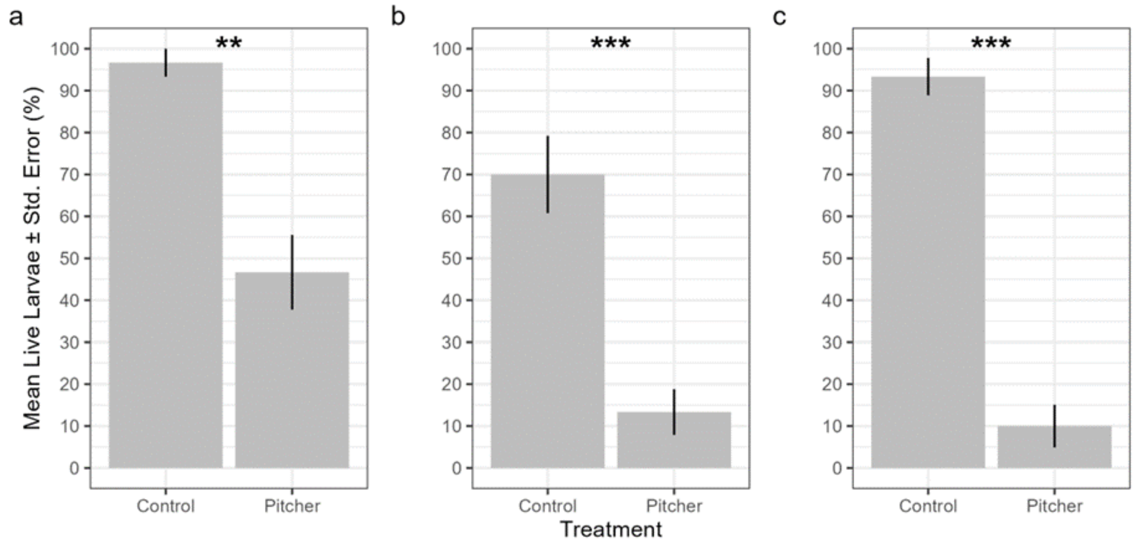
Mean Hatched Eggs ± Std. Error (%)
100
90
80
70
60
50
40
30
20
10
0
Control
Pitcher
Treatment

Figure 2


a
b
c
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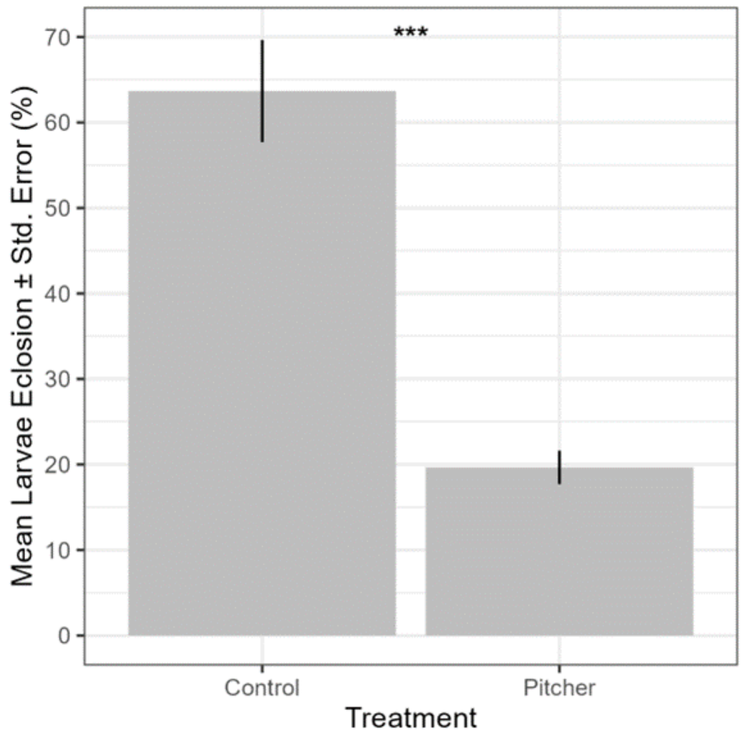
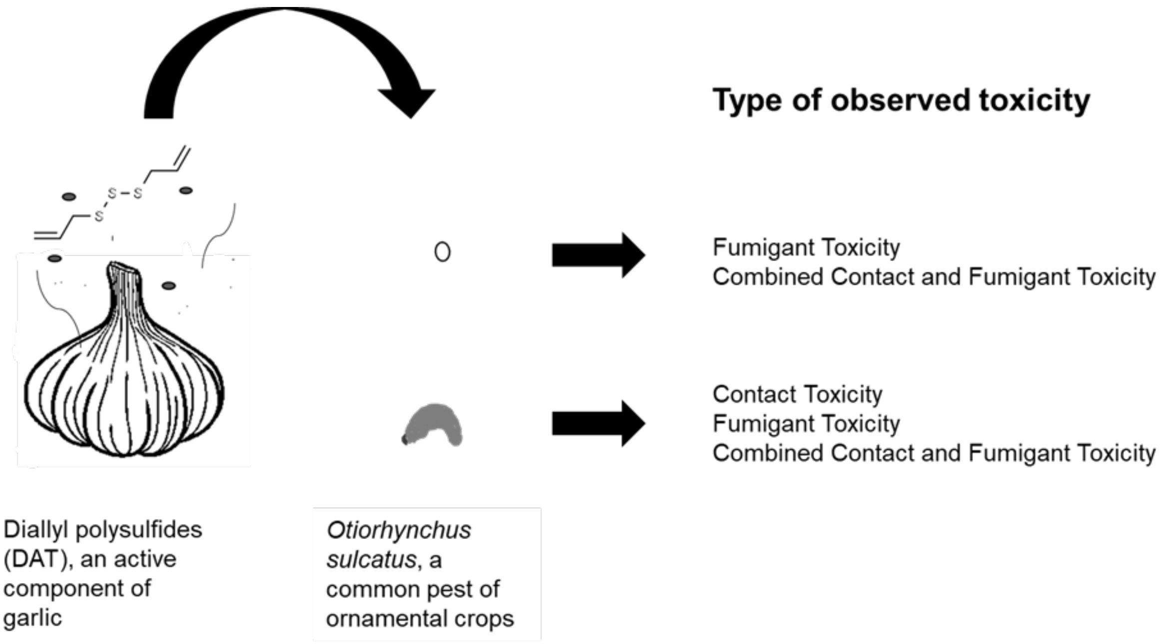
Mean Live Larvae ± Std. Error (%)
0
10
20
30
40
50
60
70
80
90
100
Control
Pitcher
Treatment

Figure 3



***
Mean Larvae Eclosion ± Std. Error (%)
0
10
20
30
40
50
60
70
Control
Pitcher
Treatment

Figure 4



Graphical Abstract Figure