

Article

Behavioural Responses of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) to Powders of Three Invasive Alien Plants, *Gleditsia triacanthos*, *Chromolaena odorata*, and *Schinus terebinthifolia*: Repellence and Locomotion Patterns

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Abstract

Although the sublethal effects of botanical pesticides are critical for pest management, their role in modifying insect behaviour is frequently overlooked. In a laboratory bioassay utilising powder prepared from the leaf, stem, and root of the invasive alien plants (IAPs) *Gleditsia triacanthos*, *Chromolaena odorata*, and *Schinus terebinthifolia*, this study evaluated the behavioural reactions, notably repellence and locomotion, of *Spodoptera frugiperda* larvae. For repellence, six second instar larvae of *S. frugiperda* were individually placed in 30 mL vial that remained unsealed to allow larvae to exit freely if repelled and were exposed to 22% of leaf, stem and root powders of all three plant species over a 12 h period before recording the number of repelled insects. For locomotion, ten-second instar larvae were directly coated with different powder quantity 1.5 g and 2.5 g of leaf, stem, and root of all three IAPs, then released and allowed to move for 60 s on a 90 × 60 cm whiteboard. The running speed was calculated as the distance travelled per unit time. All plant-part powder exhibited significantly higher repellence than the control (0%). *Gleditsia triacanthos* leaf, root and stem powders showed minimal repellence, with values of 16%, 29% and 21%, respectively. However, higher rates were observed for *C. odorata* and *S. terebinthifolia*. Specifically, *C. odorata* stem powder achieved the highest overall repellence at 54%, whereas *S. terebinthifolia* performed best with its root powder at 45%. Compared to the control group, the running speed of the insects experienced a substantial reduction, decreasing by 60 to 80% when exposed to powders derived from all parts of the three IAPs. Our results suggest that the IAP powders used in this study both repel *S. frugiperda* and strongly limit their ability to move, highlighting their potential for behaviour-based pest control strategies.



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1. Introduction

One of the important developed approaches to supporting pest control is by altering pest behaviour [1–3]. Behaviour is defined as observable actions animals do in response to internal or external stimuli [4,5]. Insect behaviour is a complex interaction influenced by

genetic factors responsible for internal responses [6–9], and environmental conditions that enable survival and adaptation in diverse ecosystems [10]. Communication, movement, mating, finding food, and habitat seeking are all a wide range of behavioural activities [11]. Contact, spatial and olfaction reception play key roles in regulating insect behaviour and have been targeted by conventional insecticides globally. However, given the environmental toxicity of modern pesticides, there is a growing interest in advocating for the biological methods of controlling insects [12,13].

Although the integration of behavioural modification has increasingly gained attention, its application in agricultural systems remains limited, despite evidence that behaviour manipulation strategies can effectively disrupt key insect functions. One study that highlights the manipulation of behaviour was done in 2017, where researchers studied the behaviour of the thrips *Megalurothrips sjostedti* Trybom (Thysanoptera: Thripidae) [14]. After understanding how thrips interact and mate, attractants were developed to trap and spread fungal biopesticide among thrips, which reduced their population effectively [15].

Apart from the use of contact attractants or repellent chemicals, other studies showed that the use of spatial chemicals plays a significant role in disrupting insect pest behaviour. For example, [16] showed that nine essential oils inflicted a significant spatial repellence to both nymphs and adults of *Halymorpha halys* Stal (Hemiptera: Pentatomidae). Cagán et al. [17] reported that the storage pests *Acanthoscelides obtectus* Say (Coleoptera: Bruchidae), *Tribolium confusum* Jacquelin du Val (Coleoptera: Tenebrionidae), and *Tenebrio molitor* Linnaeus (Coleoptera: Tenebrionidae) were significantly repelled by essential oils. The study further noted that, in addition to contact and spatial chemicals, substances affecting olfactory reception are commonly used to alter insect pest behaviour.

Sex pheromones are also effective as they inhibit males from mating with females in certain insect pests [18,19]. They proved to disrupt the breeding behaviour of the oriental fruit moth, *Grapholita molesta* Busk (Lepidoptera: Tortricidae) on stone fruits [20–22]. Deriving sex pheromones from botanicals has been explored since the 1980s, when a mixture of geraniol + phenethyl propionate (PEP) + eugenol elevated the attractants of this lure to *Popillia japonica* Newman (Coleoptera: Scarabaeidae) [23]. Other studies revealed that *Bemisia tabaci* Gennadius (Hemiptera: Aleyrodidae) adults, pests of many greenhouse crops, which were treated with nets dipped in citronella *Cymbopogon winteri* (DC) Stapf (Poaceae) and geraniol extracts had high irritant, deterrence, and repellent effect [24–26]. This shows that an improved understanding of behaviour and the role of botanicals aid the deployment of diverse sustainable pest management practices, which are beneficial for crop protection and a safer choice than the conventional synthetic chemicals.

Both internal and external factors influence behaviour, they directly affect repellence and locomotion responses as key behavioural indicators in assessing the efficacy and physiological impact of pest control agents [27]. The term repellence is commonly interpreted as pushing something away, either by odour or substances that are of nuisance and function as an irritant or deterrence [28]. The strategy behind repellents is to hinder pests from reaching essential resources by rendering the space unfavourable, altering typical behavioural patterns, and imitating a deterrent plant compound [29]. Repellents could be solids, powders, or sprays [30]. However, there are limited recent studies done on how these compounds influence insect behaviour, particularly in terms of repellence and locomotion. For effective and sustainable pest management, it is crucial to assess both lethal and sublethal effects of insecticides [31]. While lethal effects measure immediate kill rates, including effective concentrations, mortality rates, sublethal effects reveal how surviving pests may be weakened or behaviourally altered, such as affecting movement and inducing repellence [32].

Relying only on mortality may underestimate the true effect of insecticides for sub-lethal effects [33]. Even though these insects are not dead, they might not contribute much to the next generation, or might behave abnormally, reducing their threat to crops. As the emphasis is still on behavioural solutions, it is worthwhile exploring the role of repellence as an added strategy to pest control. Besides mortality, changes in movement behaviour can significantly affect an insect's ability to feed, reproduce, or escape predation [34]. Incorporating locomotion assays into pest control research provides a more complete understanding of how sublethal doses influence insect survival and ecosystem interactions.

By understanding and disrupting pest locomotion and foraging decisions, it becomes possible to limit pest spread across fields and prevent infestations from escalating. Intervening during key movement periods allows for more precisely timed control measures, enhancing their efficiency while reducing reliance on broad-spectrum chemical treatments. This study focused on three botanically diverse species with documented bioactive profiles against one of the most problematic global agricultural pests, *Spodoptera frugiperda* Smith (Lepidoptera: Noctuidae). *Gleditsia triacanthos* Linnaeus (Fabaceae), for example, contains phenolics, flavonoids, sterols, and triterpenes that have been widely utilised in other disciplines [35]. *Chromolaena odorata* (L.) King & Rob (Asteraceae) has a range of secondary phytochemicals, including alkaloids, tannins, saponins, terpenes, phenols, chalcones, steroids, flavonoids, and terpenoids, which have contributed to its recognition as a medicinal plant [36]. *Schinus terebinthifolia* Raddi (Anacardiaceae) has also been reported to contain several terpenes, phenolic compounds, tannins, alkaloids, flavonoids, and anthocyanin compounds [37,38]. Therefore, this study aimed to evaluate the behavioural responses of *S. frugiperda* to three IAPs: *G. triacanthos*, *S. terebinthifolia*, and *C. odorata* in the laboratory. This study hypothesised that extracts from *G. triacanthos*, *S. terebinthifolia*, and *C. odorata* would exhibit significant differences in repellence activity against *S. frugiperda* larvae, and that repellence would vary among plant parts (leaf, stem, and root). It was also hypothesised that exposure to different concentrations and plant parts (leaf, stem, and root) of *G. triacanthos*, *S. terebinthifolia*, and *C. odorata* would differentially affect the locomotion behaviour of *S. frugiperda* larvae. The objectives were to firstly, investigate how powder treatments (leaf, stem, and root) from these IAPs affect the repellence behaviour of *S. frugiperda* in the laboratory and secondly, to evaluate the effect of different concentrations of the plant powder treatments (leaf, stem and root) of three IAPs on locomotion behaviour of *S. frugiperda* in the laboratory.

2. Materials and Methods

2.1. Sampling Sites and Collection of Plant Materials

Various fresh plant parts of *G. triacanthos* were collected from the University of the Free State, in the Free State province (29°06'53" S, 26°11'19" E). Plant parts of *C. odorata* and *S. terebinthifolius* were collected from the Cannonbrae, Umkomaas, in Kwa-Zulu-Natal province (30°12'30" S, 30°47'12" E) (Figure 1). For each species, three individual plants were sampled, and leaf, stem, and root tissues were collected in bundles to ensure sufficient quantities were available for all analyses and to avoid shortages during laboratory procedures. The roots were excavated using a shovel, a hoe, a lasher, a pick, and a mattock. All roots near the trunk and farther away were collected, and when accessible, the entire stump was included. Secateurs and a pruning saw were used for larger and smaller stems, while healthy green leaves were collected by hand. Only healthy-looking plant parts showing no signs of disease or nutrient deficiency were collected. Sampling took place over a three-month period from February to April 2024. No specific sampling design was implemented as the choice was based solely on the availability, health, and accessibility of the invaded ecosystems. The size or age of the trees was not considered, as all plants

were used depending on their availability and accessibility. During the collection period, plants were stored in a room temperature, and a low-light area to prevent deterioration and preserve their natural chemical compounds. They were kept in boxes during transportation. After collection, all roots were washed separately with running tap water to remove any soil. All the plant parts were then evenly spread on paper on desks and left to air dry completely for two months at room temperature in a well-ventilated laboratory, away from direct sunlight [39]. The repellence and locomotion performance experiments were all conducted in the Department of Zoology and Entomology at the University of the Free State, Bloemfontein, South Africa.

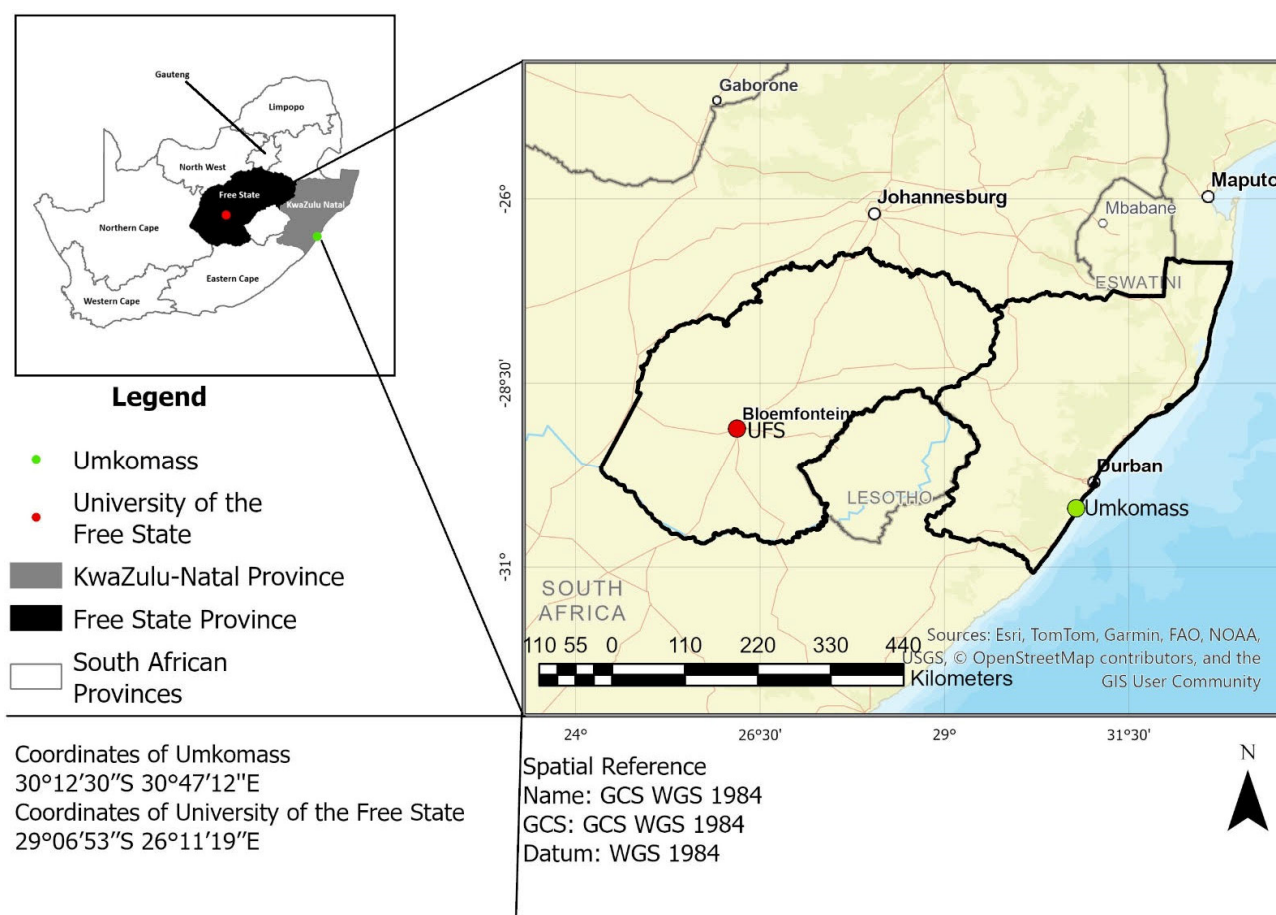


Figure 1. South African map showing sites for plant collection. Umkomass, Kwa-Zulu-Natal and the University of the Free State, Bloemfontein campus, Free State.

2.2. Processing and Preparation of Plant Powder

After air drying as described above, all stems and roots were cut into smaller pieces, measuring 5 to 10 mm, using secateurs. These pieces were then ground into a fine powder with a GM-800S1 220 V/50 Hz multifunctional grinder machine, resulting in particles approximately 0.01 to 1.0 mm in size [39], purchased from Feral Foods (Pty) Ltd., 40 Swart Drive, President Park AH, Gauteng 1685, South Africa. The ground plant materials were subsequently sieved to obtain a uniform particle size distribution prior to use in the bioassays. The pulverisation process took place in the Indigenous Knowledge Systems laboratory within the Department of Pharmacology, situated at the University of the Free State, Bloemfontein Campus, South Africa. The leaves were also ground into a fine powder, using a Kenwood Multipro Express all-in-one food processor in the laboratory. After grinding, all plant materials were stored separately in airtight, dark, and waterproof containers for later use in bioassays.

2.3. Transport and Storage Processes of *S. frugiperda*

Spodoptera frugiperda neonates were purchased from the Agricultural Research Council (ARC) Insect Ecology laboratory in Pretoria, South Africa and transported to the study site in insulated containers under controlled temperature conditions ($20\text{--}28\text{ }^{\circ}\text{C}$), $80 \pm 5\%$ relative humidity to minimise stress during transportation. Upon arrival, larvae were maintained in a laboratory environment at $20\text{--}28\text{ }^{\circ}\text{C}$, $65 \pm 5\%$ relative humidity, and a 12:12 h (light) photoperiod. Larvae were grown on an artificial diet supplied by the ARC and kept in ventilated 30 mL vials sealed with cotton wool to ensure adequate aeration and prevent desiccation. All handling procedures were conducted carefully to maintain larval viability and uniform physiological condition prior to experimentation.

2.4. Repellence Bioassay on *S. frugiperda*

The experiment to determine the repellence response of *S. frugiperda* was conducted at an ambient temperature of $28 \pm 2\text{ }^{\circ}\text{C}$ and $65 \pm 5\%$ RH in the biology building in the insectarium. Before the repellence bioassay began, a preliminary assessment was conducted to find the effective concentration range, but only one standard concentration was used to conduct the actual experiment throughout different treatments. A preliminary trial evaluated five concentrations. Concentrations below the 3.0 g produced no effect, hence 3.0 g was used in the main study. To evaluate repellence in the main trial control, leaf, stem, and root powders of *G. triacanthos*, *S. terebinthifolius*, and *C. odorata*, plant species were used against *S. frugiperda* larvae. For the repellence bioassay, each plant powder measuring 3.0 g was incorporated directly into the 10.53 g artificial diet and was mixed to create a plant-part mixed diet treatment, making a 22% *w/w* ratio, calculated as the weight of the plant powders relative to the weight of the artificial diet, while the control diet received no plant powders. Plant powders were each mixed in a Petri dish, then transferred into a vial measuring 30 mL. Six second-instar *S. frugiperda* larvae were individually introduced into each vial through an opening. The containers were placed inside a fully enclosed, transparent rectangular 5 L plastic container measuring $34 \times 20 \times 13\text{ cm}$, allowing correct counting of larvae exiting the treated diets in the 30 mL vials into the 5 L transparent container. Each treatment was replicated four times, and the larvae were exposed for a total period of 12 h. A post hoc power analysis confirmed that our design achieved a statistical power of between 97.7% and 98.5%, vastly exceeding the standard academic threshold of 80% and validating the adequacy of our replication level of four ($n = 4$) per plant type. Control treatments consisted of diets not treated with any IAP powders and were checked over the same periods. The number of insects leaving the treated diets served as a measure of repellence for the different powders. A total of 60 insects were used, following [40] with modifications.

2.5. Locomotion Performance Trial of *S. frugiperda*

Before the experiment, the larvae were placed in a 30 mL vial in preparation for the trial. The effect of leaf, stem, and root powders of *G. triacanthos*, *C. odorata*, and *S. terebinthifolius* plants on *S. frugiperda* larvae locomotor ability was determined by recording the speed of movement and distance covered in 60 s when treated with 1.5 g and 2.5 g of plant powders. From the range-finding test, concentrations of 1.5 g and 2.5 g were selected based on effectiveness. Doses below 1.5 g failed to produce measurable activity, while those above 2.5 g did not enhance the response further. These quantities, therefore, represent the most informative points for evaluating the plant-part powders. Ten *S. frugiperda* larvae were exposed individually to different quantities of the test plant-parts by applying the powder directly to the larvae in the vial and kept in there for five min to acclimate, after which the insect was allowed to walk on a whiteboard of $90\text{ cm} \times 60\text{ cm}$ for 60 s. An HB

pencil was used to trace the distance travelled by the larvae. Because the larvae travelled in a zigzag manner after treatment with plant-part powder, a thread was used to measure the distance walked by the larvae and the length of the thread was measured with a ruler and recorded. A total of ten replicates were used for each concentration of the plant-part powder. A total of 190 larvae were used. The speed of individual *S. frugiperda* larvae was calculated by dividing the distance covered (cm) by the total time the larvae were exposed (s) [41]. The experiment to determine locomotion behaviour of *S. frugiperda* was conducted at an ambient temperature of 28 ± 2 °C and $65 \pm 5\%$ RH in the biology building in the insectarium.

2.6. Data Analysis

We utilised the arcsine square-root transformation on our percentage repellence data prior to analysis because the raw proportions violated the standard assumptions of normality and homoscedasticity required analysis. After Shapiro–Wilk and Levene’s tests were used to confirm that the assumptions of normality and equal variance were successfully satisfied, the effect of plant powders (leaf, stem, root and control) from three different invasive alien plants on insect repellence, was analysed with Generalised Linear Model (GLM) assuming a normal distribution with an identity link function. Following arcsine square root transformation of the locomotion data, the effect of the different quantities of the powders (leaf, stem and root) of three invasive alien plants on the locomotion performance of *S. frugiperda* was analysed using Generalised Linear Model (GLM) assuming a normal distribution with an identity link function. When the overall results were significant in the GLM analysis, the difference among the treatments was compared using the Sequential Bonferroni test. All the data were analysed using SPSS Statistical software, version 16.0 (SPSS, Chicago, IL, USA).

3. Results

3.1. Repellence of *S. frugiperda* by *G. triacanthos*, *C. odorata*, and *S. terebinthifolia*

Plant powders from three different parts of *G. triacanthos* exhibited some repellent activity against *S. frugiperda* compared to the control treatment (Figure 2). Following a 12 h exposure of *S. frugiperda* to powder from different plant parts, percentage repellence significantly differed (GLM: Wald ($\chi^2 = 18.129$, $p = 0.001$). Among the treatments, the control exhibited zero repellent activity (0.0%) against the *S. frugiperda*. In contrast, the leaf and stem powders increased repellence to 16% and 21%, respectively, while the root powder exhibited the highest repellence activity, 29% (Figure 2).

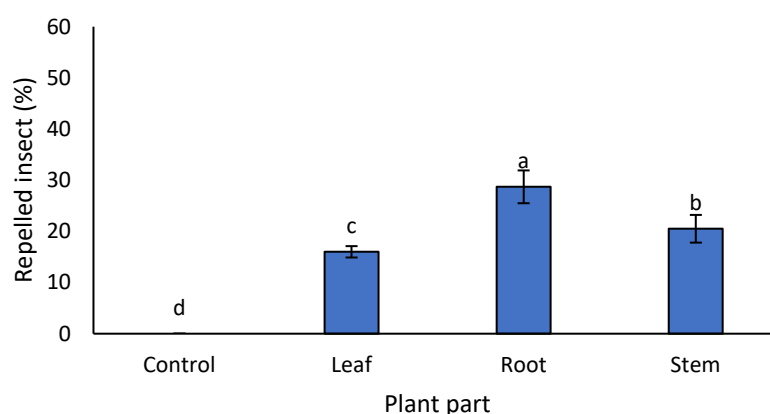


Figure 2. Percentage mean ($\pm$ SE) repellence of different plant treatments, of *G. triacanthos* powder, against *S. frugiperda* exposed for 12 h. Means capped with different letters are significantly different (Sequential Bonferroni’s test $p < 0.05$).

Plant powders from three different parts of *C. odorata* exhibited some repellent activity against *S. frugiperda* compared to the control treatment (Figure 3). Following a 12 h exposure of *S. frugiperda* to powder from different plant parts, the percentage repellence significantly differed ($\chi^2 = 28.766$, $p = 0.001$). Among the treatments, the control exhibited zero repellent activity (0.0%) against the *S. frugiperda*, while the leaf and root exhibited 37.2% and 28.5% repellence, respectively. Stem powder exhibited the highest repellent activity, 54% (Figure 3).

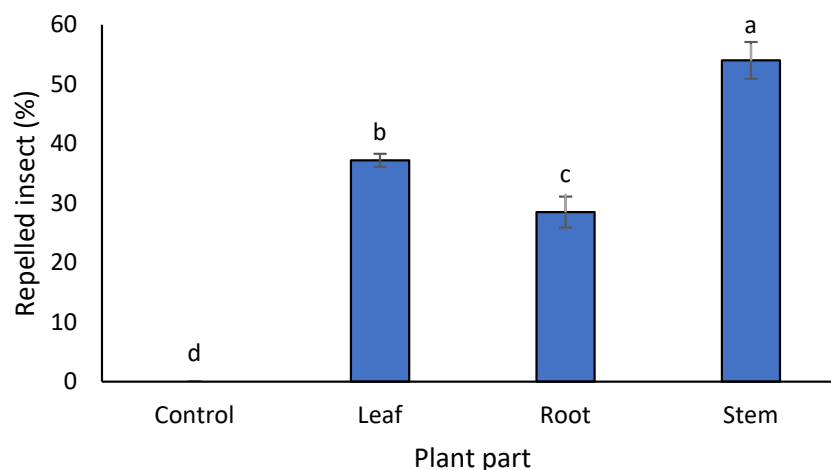


Figure 3. Percentage mean ($\pm$ SE) repellence of different plant treatments, of *C. odorata* powder, against *S. frugiperda* exposed for 12 h. Means capped with different letters are significantly different (Sequential Bonferroni's test $p < 0.05$).

Plant powders from three different parts of *S. terebinthifolia* exhibited some repellent activity against *S. frugiperda* compared to the control treatment (Figure 4). Following a 12 h exposure of *S. frugiperda* to powder from different plant parts, the percentage repellence significantly differed ($\chi^2 = 27.280$, $p = 0.001$). Among the treatments, the control exhibited no repellent activity (0.0%) against *S. frugiperda*. In contrast, the leaf and stem showed 24.7% and 24.5% repellence, respectively, while the root powder exhibited the highest repellent activity, 46% (Figure 4).

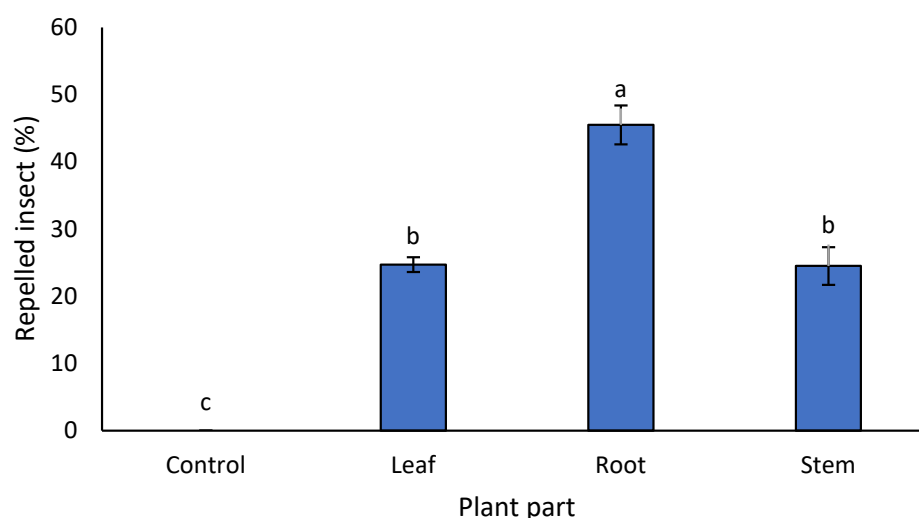


Figure 4. Percentage mean ($\pm$ SE) repellence of different plant treatments, of *S. terebinthifolia* powder, against *S. frugiperda* exposed for 12 h. Means capped with different letters are significantly different (Sequential Bonferroni's test $p < 0.05$).

3.2. Locomotion Performance of *S. frugiperda* Exposed to Powders of *G. triacanthos*, *C. odorata*, and *S. terebinthifolia*

Locomotion performance of *S. frugiperda* varied as a function of *G. triacanthos* (plant-powder quantity) ($\chi^2 = 240.364$, $p = 0.0001$) but not as a function of plant part ($\chi^2 = 0.404$, $p = 0.817$). The running speed of *S. frugiperda* varied significantly, with larvae exposed to the control treatment running the highest speed (0.41 cm/s) compared to all plant-part treatments (Figure 5). *Spodoptera frugiperda* running speed was reduced by more than 0.25 cm/s when exposed to 1.5 g of the three plant powders, and about 0.29 cm/s when exposed to 2.5 g of the plant powders. Overall, the plant powders reduced the locomotor performance of *S. frugiperda* by more than 60%. There was no significant interaction between powder quantity and plant parts ($\chi^2 = 0.289$, $p = 0.991$), showing that the effect of powder quantity on *S. frugiperda* running speed did not differ across the three plant parts.

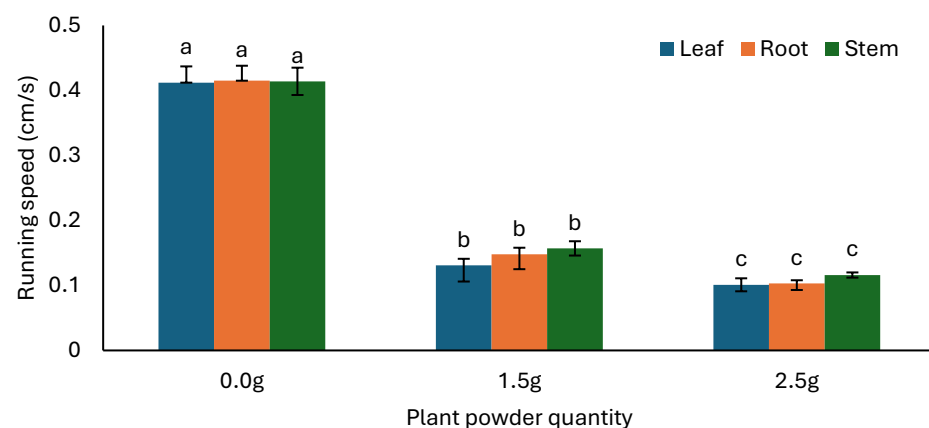


Figure 5. Mean ($\pm$ SE) running speed of *S. frugiperda* after exposure to different powder quantities of *G. triacanthos* plant parts (leaf, stem, root). Means capped with different letters are significantly different (Sequential Bonferroni's test $p < 0.05$).

Locomotion performance of *S. frugiperda* varied as a function of *C. odorata* plant powder quantity ($\chi^2 = 286.364$, $p = 0.001$), but not as a function of plant part ($\chi^2 = 0.762$, $p = 0.683$). The running speed of *S. frugiperda* larvae varied significantly, with those in the control treatment (no plant powder) exhibiting the highest speed at 0.42 cm/s. In contrast, the treatments markedly reduced larval running speed by approximately 0.34 cm/s, representing over 80% reduction in the running speed of *S. frugiperda* (Figure 6). There was no significant interaction between plant powder quantity and plant parts ($\chi^2 = 1.015$, $p = 0.908$), indicating that the concentration was consistent across the various plant parts.

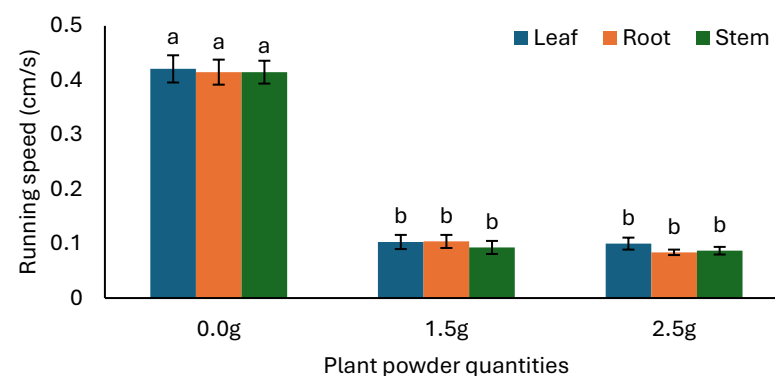


Figure 6. Mean ($\pm$ SE) running speed of *S. frugiperda* after exposure to different quantities of *C. odorata* plant parts (leaf, stem, root). Means capped with different letters are significantly different (Sequential Bonferroni's test $p < 0.05$).

Locomotion performance of *S. frugiperda* varied as a function of *S. terebinthifolia* plant powder quantities ($\chi^2 = 255.514$, $p = 0.001$), but not as a function of plant part ($\chi^2 = 0.948$, $p = 0.623$). The running speed of *S. frugiperda* larvae varied significantly, with those in the control treatment (no plant powder) exhibiting the highest speed at 0.41 cm/s. In contrast, the plant powders markedly reduced larval running speed by approximately 0.29 cm/s, representing a percentage speed reduction of 70% (Figure 7). There was no significant interaction between plant powder quantity and plant parts ($\chi^2 = 0.895$, $p = 0.925$), showing that the concentration was consistent across the three plant parts.

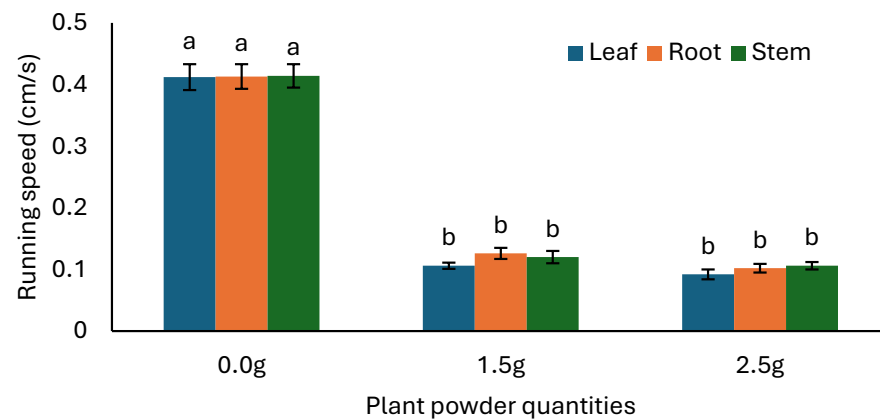


Figure 7. Mean ($\pm$ SE) running speed of *S. frugiperda* after exposure to different powder quantities of *S. terebinthifolia* plant parts (leaf, stem, root). Means capped with different letters are significantly different (Sequential Bonferroni's test $p < 0.05$).

4. Discussion

4.1. Repellent Effects of *G. triacanthos*, *C. odorata*, and *S. terebinthifolia* Plant Powders on *S. frugiperda*

Sublethal, behaviour-modifying pest control methods are under-researched, and in South Africa, particularly, IAPs remain largely unexplored as potential sources of bio-insecticidal compounds [42,43]. This study indicated that the sublethal effect from IAPs substantially repelled *S. frugiperda* larvae and significantly reduced larval locomotion ability. Furthermore, this study indicates that exploring the response of *S. frugiperda* to sublethal doses of IAP powders, especially in repellence and locomotion performance, may offer an additional technique to reduce pest infestation and crop damage. In this study, repellence was first evaluated to see if the plant powders could keep *S. frugiperda* larvae away from important crops, which were represented by an artificial diet in the experiment. Secondly, if not repelled, the locomotion of *S. frugiperda* was assessed to see how much they can move between crops and cause damage by investigating larval running speed following the application of the plant-based treatment. These findings may help maize producers to better understand the behaviour of *S. frugiperda* larvae to sublethal doses of IAP powders.

Gleditsia triacanthos (leaf, stem and root) powders exhibited the lowest repellence effect against *S. frugiperda* larvae compared to other plant species. However, the plant powders significantly repelled the *S. frugiperda* larva compared to the control. Even though much attention and interest are given to the plant part (roots) that repelled more larvae, it is equally important to evaluate the performance of other plant parts (leaf and stem), as this can inform future studies and give information on their mechanism of action [44]. A total of 29% larvae were repelled from treatment with *G. triacanthos* roots, followed by the stem, 21%, and then the leaf, which only repelled 16% of the larvae. The control group showed 0% repellence, showing that no disturbance occurred, as normal activity was seen, where larvae kept on feeding on the diet. This confirms that the treatments from IAPs had a

measurable effect. Previous studies have reported that root tissues of some plant species may contain bioactive compounds, including essential oils and other volatile constituents, which can influence insect behaviour. Therefore, the moderate repellence observed in root powders in the present study may be associated with such compounds [45]. Although root powder showed the most promising results with the highest observed repellence, unfortunately, it is regarded as moderate because 70% of the larvae were not repelled and still pose a threat to crops. Therefore, this laboratory experiment suggests that *G. triacanthos* is not practical enough to repel and protect the crops from *S. frugiperda* larvae in the field. Studies indicated that increasing powder doses, understanding their mode of action, or using them in different forms, such as extracts, could improve their effectiveness [46].

Chromolaena odorata powders exhibited the highest repellence among the three invasive alien plant species evaluated in this study. This finding is consistent with previous studies conducted in Nigeria, which have also reported strong repellent properties of *C. odorata* against insect pests [29,40]. All plant-part powders significantly repelled the larvae, with the root powder being the least effective at 29%, followed by the leaf at 37%. The stem repelled 54% of the larvae, indicating a potentially strong production of secondary metabolites or toxins that may have deter most larvae due to richness in volatile compounds. Research on *C. odorata* reveals that it contains a high content of saponins, while phytate and tannin levels are moderate [47], and this may explain the stem efficacy compared to other plant parts. Although studies on the spectrum of bioactive chemical compounds produced by *S. terebinthifolia* roots and *C. odorata* stems are limited, a similar study conducted by [48] reported high repellence from *C. odorata* stem powders, potentially due to the presence of toxic bioactive compounds present in plant species [49].

Lastly, the *S. terebinthifolia* plant-part powders showed better performance over *G. triacanthos* plant-part powders with better repellent efficacy. Although there was no significant difference between the leaf and the stem, which both repelled 25% of the larvae, this was still significant compared to the control, where 100% of the larvae were not repelled. The root powder demonstrated the highest repellence among the tested plant powders, affecting nearly 50% of the larvae. As reported in previous studies [50], plant tissues may differ in their physiological and metabolic characteristics, such as production of primary and secondary metabolites.

Although volatile monoterpenes were not isolated in the present study, previous reports indicate that such compounds may occur in plant tissues and could potentially be associated with the observed repellence [51]. Other studies have shown that conventional toxic repellents often prove ineffective at low concentrations and only become effective at higher doses, which can lead to environmental toxicity and harm to non-target organisms [52,53]. Conversely, this study's findings indicate that plant-based alternatives are effective against *S. frugiperda* under laboratory conditions, warranting further investigation for pest management applications. Notably, if *C. odorata* stem treatment achieves repellence rates exceeding 50% at low concentrations, it represents a significant breakthrough in biological repellence. These findings suggest that the powder has the potential to reduce pest populations, potentially addressing up to half of the infestation under the tested conditions. This level of efficacy indicates that the powder could reduce reliance on synthetic insecticides, thereby lowering control costs. Such levels of effectiveness suggest practical viability for protecting crops in agricultural environments, especially where chemical repellents are undesirable. These plant materials from *G. triacanthos*, *S. terebinthifolia*, and *C. odorata*, present potential for further investigation as bio-repellents for *S. frugiperda* management [54]. The aim was not only to reduce feeding but also to prevent insects from locating their hosts and oviposition sites. Therefore, this study focused on preventing contact with the plant before damage occurs [27,55].

4.2. The Effect of *G. triacanthos*, *C. odorata*, and *S. terebinthifolia* Powders on the Locomotion of *S. frugiperda*

From this study, locomotion performance of *S. frugiperda* varied depending on the concentration of plant powders. Out of the three plant powder quantities assessed, two (1.5 g and 2.5 g) significantly reduced speed by 50%, which is less than 0.2 cm/s across all plant parts, although performing similarly across these varying quantities. The reduced locomotor speed (approximately 50% of normal) indicates potential impairment in motor neuron function, muscular activity, or energy metabolism [56]. In the field, 50% reduction is considered substantial and normal activities, including spreading and feeding, may be reduced [57]. Larvae that were treated with no plant powder (0.0 g) in the control group showed the highest speed of 0.42 cm/s across different treatments, indicating that these insects may have kept high energy levels and that their physiology was not affected by any plant exposure [58]. The over 60% reduction in the running speed of *S. frugiperda* larvae by all plant parts powder suggests that the plant materials may possess strong locomotor inhibitory properties, which may interfere with neuromuscular coordination [59]. This consistent effect across all plant parts indicates that bioactive compounds might be widely distributed within the plants and may be capable of significantly impairing larval locomotion.

A previous study reported that *S. frugiperda* larvae can move to other parts of the plant at a speed of about 0.05 cm/s [60], but wind and silk threads can enhance dispersal [61–63]. Untreated larvae can cover notable distances in 60 s, evade control, and reduce pest management success, increasing crop losses and food security risks [64,65]. Reducing movement increases vulnerability to predators and interventions, limiting spread and survival [66]. Locomotion performance influences insect pest migration and dispersal [67,68], which depends on insects' energy metabolism and environmental factors [69,70]. Insects use energy to move for feeding and reproduction [71,72]. Since environments cannot be controlled, reducing pests' energy is key. Speed enhances survival, resource finding, predator avoidance, mating, and rapid spread [73,74].

Hassan et al. [75] demonstrated that *Metarhizium anisopliae* infection in *Solenopsis invicta* reduced locomotion (shorter distance, lower velocity, increased turning), impaired foraging, altered stress and neurotransmission-related enzymes, and decreased survival, indicating systemic physiological effects. In the present study, *G. triacanthos* leaf, stem, and root treatments showed no significant differences in *S. frugiperda* locomotion, and no interaction with powder quantity, indicating a consistent dose response. However, leaf extracts consistently produced the lowest mean speeds, suggesting a marginal, non-significant effect that requires further investigation.

Chromolaena odorata root powder with increased powder quantity slightly reduced the speed more than other plant-part powders. The absence of a significant difference between medium (1.5 g) and high (2.5 g) powder quantities from leaf, stem and root may suggest a plateau, showing that increasing the dosage beyond a certain point offers no added behavioural suppression. Overall, the lack of significant interaction between powder quantity and treatments implies that all three plant parts might be responding similarly to changes in quantity. In other words, no plant part showed a stronger response at a specific concentration level.

The present findings on *S. terebinthifolia* leaf powder align with previous observations that plant-derived compounds can significantly affect larval behaviour. Higher quantities of the leaf powder reduced larval speed more markedly than other plant parts, indicating that leaves may contain more potent bioactive compounds. This is consistent with the study by [76], in which methanol extracts of *Cerbera odollam* Gaertn (Apocynaceae) leaves significantly decreased the locomotion and exploratory activity of *S. frugiperda* larvae,

with higher concentrations causing pronounced reductions in movement and increased periods of immobility. These studies suggest that leaf-based botanical treatments may effectively disrupt larval behaviour, since insects typically avoid unpalatable or chemically contaminated food sources [77], potentially reducing feeding activity and limiting pest spread [78]. The present study also observed that *S. frugiperda* larvae coiled up, remained motionless, and exhibited minimal movement following each treatment. These behavioural responses were accompanied by significant differences in repellence among root, stem, and leaf powders, likely attributable to variations in the concentration and composition of bioactive compounds across plant parts, while the lack of significant differences in running speed indicates a consistent locomotor response of insects under the tested conditions. Although locomotion was measured using manual tracing, repeated observations and careful measurement procedures were employed to minimise potential measurement errors and ensure consistency across treatments. Nevertheless, future studies could benefit from automated video-tracking systems or other technologies to provide more precise and objective assessments of insect movement.

From an agricultural standpoint, examples of effective control strategies using repellent and locomotion behaviour manipulation in integrated pest management (IPM) are scarce compared to those using insecticides. The results suggest careful consideration of management of *S. frugiperda* using IAPs, which has many promising advantages, including reduced use of synthetic chemicals. Fewer studies in South Africa have demonstrated the ability of plant-based materials to successfully affect behavioural responses in targeted pests, highlighting their potential for incorporation into alternative pest management strategies, compared with synthetic pesticides [79–81]. Plant samples were collected based on field availability to reflect a realistic and practical approach to the use of invasive alien plant species as locally accessible resources for pest management. However, a limitation of this study is that plant age, size, and phenological stage were not controlled, which may influence the concentration of secondary metabolites in different plant parts. Future studies should therefore consider standardised sampling across defined growth stages and seasons, as well as phytochemical characterisation of the active compounds responsible for the observed behavioural effects.

5. Conclusions

This study demonstrates that plant powders derived from *G. triacanthos*, *S. terebinthifolia*, and *C. odorata* can influence the behaviour of *S. frugiperda* larvae by inducing moderate repellence and reducing locomotor activity. Although there were no significant differences among the three plant species, all treatments consistently reduced larval movement, suggesting a potentially useful behavioural disruption effect. This is particularly important given the global threat posed by *S. frugiperda*, which causes extensive crop damage and economic losses and has developed resistance to many conventional chemical insecticides. These findings support the growing need for sustainable and environmentally compatible pest management strategies. Behavioural interference, especially through repellence and reduced mobility, may offer a promising non-lethal approach that could complement integrated pest management systems.

However, the study has several limitations. The experiments were conducted exclusively under laboratory conditions without field validation, and no phytochemical analysis was performed to identify the active compounds responsible for the observed effects. In addition, larval movement was measured manually, which may introduce minor measurement bias despite careful procedures. Future research should therefore include field trials to confirm real-world effectiveness, alongside chemical profiling to identify the bioactive constituents involved. The use of automated tracking systems would also improve the

precision and objectivity of behavioural measurements. Furthermore, safety assessments on non-target organisms, environmental impact, and potential human health risks are essential before practical application can be recommended. Overall, this study provides preliminary but promising evidence that invasive plant species may serve as a basis for developing novel, behaviourally based pest management tools against *S. frugiperda*.

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Abbreviations

The following abbreviations are used in this manuscript:

IAP	Invasive Alien Plants
ARC	Agricultural Research Council
RH	Relative Humidity

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