PROJECT NO. 51-2022
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PROJECT TITLE: Sicklepod extract formulations as natural and effective deer and insect repellent

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EXECUTIVE SUMMARY

This study aimed to evaluate the efficacy and compatibility of a sicklepod extract-herbicide tank-mix for deer and insect management in soybean crops. Field trials were conducted over multiple quarters, involving various treatments and measurements. The field trials examined the impact of different herbicide combinations on deer browsing and insect populations in soybean plots. Weeds were found to interfere with deer browsing, as glyphosate-treated plots without weed cover experienced the most damage. Insect data collection revealed the presence of leaf-chewing insects and other bugs.

The study also investigated the efficacy of coffee senna and hemp sesbania extracts in preventing insect feeding in soybean. The extracts demonstrated promising results in causing mortality among soybean loopers. Additionally, the efficacy of prickly sida extract was tested and found to result in a high mortality rate for soybean loopers. However, when the experiments were repeated using stored extracts, the mortality rates varied, potentially due to extract decay.

Furthermore, the study explored the impacts of sicklepod extracts on non-target beneficial bee pollinators. Bee experiments were conducted, focusing on the transportation of anthraquinone, a compound found in sicklepod, within soybean plants. Anthraquinone was detected in the shoot tips of treated plants, indicating its transport through the vascular system. Anthraquinone concentrations in soybean flowers initially showed higher levels in treated plants, which rapidly decreased over time. Anthraquinone was not detected in the shoot tips of untreated plants, suggesting a localized distribution within the flowers and the potential involvement of unknown pollinators.

Overall, this study provides valuable insights into the efficacy and compatibility of the sicklepod extract-herbicide tank-mix for deer and insect management in soybean crops. The findings emphasize the importance of weed extracts in deer browsing and highlight the complex interactions between treatments, pests, and beneficial organisms. The study also sheds light on the transportation and distribution of anthraquinone in soybean plants and its potential implications for non-target beneficial bee pollinators. The results contribute to a better understanding of agricultural practices and ecological considerations in soybean cultivation.

BACKGROUND AND OBJECTIVES

White-tailed deer are responsible for 70% of the wildlife caused crop losses totaling $4.5 billion in crop losses each year (Miller et al. 2015). Soybean plants protected from white-tailed deer browsing were 25% taller, 87% less damaged, yielded 74% more seed, and had 47% more above-ground biomass than unprotected plants (previous MSPB project). A loss of $68/ha or a 43% financial loss over one growing season to deer browsing is estimated (Conover 2002). Unfortunately, the only effective and widely used technique to control deer in soybean currently is the establishment of fences or application of repellents (Cauteren et al. 2006). Fencing is expensive and labor-intensive to install and require weekly inspection and maintenance throughout the growing season to ensure effective operation and longevity (Ward et al. 2010). The effectiveness of repellents is also reduced by rainfall that may dissolve repellents requiring reapplication and are not effective against deer. Moreover, soybean insect pests, especially soybean aphids and loopers, have the potential to reduce soybean yield by up to 41% and the number of pods per plant by up to 40% when present at the R2 stage at a density of 100 aphids/plant.

Plants possess varying levels of herbivore defense mechanisms, and weeds, because of their vast genetic and phenotypic diversity, are excellent resources for anti-herbivore traits (especially against deer and insect pests). No one has tried to test the activity and effectiveness of these anti-herbivore compounds or plant extracts on crop protection. In our previous studies funded by MSPB, we conducted tests at the Captive Deer Facility and at The R. R. Foil Plant Science Research at MSU to confirm the anti-herbivore property of sicklepod weed extracts. Sicklepod extracts were tested by application on soybean plants in a diet selection trial on captive deer and insects. Soybean plants not applied with sicklepod extract were consumed completely, while plants with sicklepod extract were entirely avoided. Moreover, the sicklepod extract had no adverse effect on the overall soybean yield. Insect damage was apparent in our treatment area, and our data suggest that soybean plants treated with sicklepod extract had lesser insect damage than two other insect repellants available in the market. Our preliminary leaf disc insect feeding results indicate that sicklepod extract and Bifen (synthetic insecticide) had similar antifeedant effects, with both exhibiting only 2% feeding, while neem oil and control showed 34 and 38% feeding, respectively. The antifeedant effect of sicklepod extract and Bifen were similar, while the control and neem oil treatments had lower and similar antifeedant effects.

The study aimed to prepare and characterize four formulations of sicklepod extract for improved deer and insect repellency. Trials in a captive deer facility and leaf-disc assays were conducted to determine the most effective formulation for repelling deer and insects. Field trials were carried out to evaluate the efficacy and compatibility of sicklepod extract-herbicide tank-mix on deer and insect management in soybean. The effectiveness of coffee senna and hemp sesbania extracts in preventing insect feeding in soybean was also evaluated. Additionally, the study aimed to identify any impacts of sicklepod extracts on non-target beneficial bee pollinators.

ACTIVITY/PROGRESS

Objective 1: Prepare and characterize four formulations of sicklepod extract for improved deer and insect repelling efficacy

In Year 1, the objective was to prepare and characterize four sicklepod fractions (A, B, C, D, and E) with improved deer and insect repelling efficacy. The study utilized leaf disc assays to assess the effectiveness of these fractions in reducing soybean looper feeding. The results indicated that fractions D and C demonstrated the highest efficacy, with reductions of 80% and 52%, respectively, compared to the control (Fig. 1). Additionally, insects fed with soybean leaves treated with fractions D and C were the smallest in size (Fig. 1), further supporting their repellent properties. Furthermore, chromatography analysis using High-Performance Liquid Chromatography (HPLC) confirmed that fractions D and C exhibited the highest anthraquinone concentrations, with levels of 15 ppm and 8 ppm, respectively. This analysis validated the insecticidal properties of anthraquinone and its contribution to the repellent efficacy of the sicklepod fractions. However, the study also identified a limitation of the current sicklepod extract formulation, which is its high viscosity. The high viscosity poses challenges in formulating an extract with a high anthraquinone concentration (>300 ppm). Increasing the anthraquinone concentration of the extract leads to an increase in viscosity, making it difficult to apply through spray booms. Sicklepod seeds contain significant amounts of galactomannans, a polysaccharide that increases the viscosity of solutions, particularly when heated. Therefore, there is a critical need to enhance the efficacy of the sicklepod extract by increasing the active ingredient concentration (anthraquinone concentration to >300 ppm) while decreasing the viscosity to ensure proper spray application. In Year 1 experiments, sicklepod seeds extracted in methanol (20%) or ethyl acetate (10%) yielded high anthraquinone concentrations (>200 ppm) and demonstrated effective leaf antifeedant effects against soybean loopers (Fig. 3). These findings suggest alternative extraction methods that can achieve higher anthraquinone concentrations and potentially overcome the issue of high viscosity in the extract formulation.

Objective 2: Use the four formulations (from objective #1) for trials in the captive deer facility, and leaf-disc assay to determine which formulation(s) is most effective in repelling deer and insect, respectively

Year 1 field trials were conducted to evaluate the efficacy of the sicklepod formulations in repelling deer and insects in soybean crops. The sicklepod formulation was compared against Hinder (a commercial deer repellent) and a control (water). The results indicated that the sicklepod formulation was the most effective in repelling deer compared to the commercial deer repellent and the control (Fig. 2). This finding is significant as deer were identified as the dominant pest in soybean production in Mississippi in June, while insects became the dominant pests in July, August, and September. Observations of deer browsing patterns revealed that in June, deer browsing extended from the plot edges to the center of soybean plots, resulting in defoliation and a reduction in soybean height (Fig. 4). The browsing was categorized into two types: browsing above cotyledons (Category A) and browsing below cotyledons (Category B). Soybean plants in Category A could still branch from cotyledons and survive, while those in Category B would die, leading to seedling loss. These losses, often randomly distributed and difficult to replace, contribute to yield reduction. Based on lessons learned from previous deer repellent experiments in agricultural fields in 2020, the sicklepod extract and sicklepod extract with glyphosate were sprayed at the V2 stage, which resulted in better protection of soybean plants from deer browsing compared to spraying at the V5 stage at the optimum planting date in late May 2021 (Fig. 5).

In Year 2, studies conducted in 2020 demonstrated that the sicklepod extract tank-mixed with glyphosate did not adversely affect soybean yield, as the yields between glyphosate and glyphosate+sicklepod extract treatments were similar. In the 2022 project, further investigations were planned to confirm the effects of sicklepod-herbicide tank-mix (glyphosate and dicamba) on weed, deer, and insect control. Regarding insect repellency, leaf disc insect feeding assays showed that the sicklepod extract and Bifen (synthetic insecticide) had similar antifeedant effects, exhibiting only 2% feeding, while neem oil and the control demonstrated 34% and 38% feeding, respectively (Fig. 8, 9). Coffee senna and hemp sesbania extracts also displayed significant mortality of soybean loopers at the 2nd and 4th instar, 24 hours after exposure to soybean leaves treated with these extracts, suggesting their potential as insect repellents (Fig. 10). The comparison between anthraquinone concentrations and looper biomass led to the conclusion that anthraquinone derivatives are the active ingredients of the sicklepod extract as a looper antifeedant (Table 1).

Objective 3: Field trials to determine the efficacy and compatibility of sicklepod extract-herbicide tank-mix on deer and insect management in soybean.

In Year 3, field trials were conducted at the Pontotoc site and the Starkville soybean deer plot to assess the efficacy of a sicklepod extract-herbicide tank-mix in managing deer browsing and controlling insect populations in soybean crops (Fig. 11). The study included eight treatments: coffee senna extract, dicamba, Deer Pro, glyphosate, sicklepod extract, sicklepod extract + dicamba, sicklepod extract + glyphosate, and a water control. Observations of deer browsing patterns at the Pontotoc and Starkville sites highlighted the role of weed interference. Plots treated with glyphosate, which effectively controlled weeds, suffered the most damage from deer browsing (Fig. 12, 13). The absence of weed cover exposed soybean plants directly to deer browsing. In contrast, sicklepod extract-treated plots exhibited low browsing, indicating the repellent effect of the extract and the protective role of weeds in reducing vulnerability to deer browsing. Shake cloth data provided insights into populations of leaf-chewing insects, including velvetbean caterpillars, green cloverworms, soybean loopers, stink bugs, and kudzu bugs (Fig. 14). The efficacy of sicklepod, sesbania, coffee senna, and prickly sida extracts on insect mortality was evaluated (Fig. 15, 16). The second soybean plot treated with these extracts showed varying levels of mortality, with coffee senna extract achieving the highest rates.

Objective 4: Efficacy of coffee senna and hemp sesbania extracts in preventing insect feeding in soybean.

In Year 3, the efficacy of coffee senna and hemp sesbania extracts in preventing insect feeding on soybean plants was assessed. The extracts resulted in the mortality of soybean loopers, indicating their potential for insect management. Prickly sida extract also demonstrated a significant reduction in soybean looper populations after exposure to treated soybean leaves for 24 hours, suggesting its effectiveness as a management tool. Comparisons between different extracts (sesbania, coffee senna, sicklepod, and prickly sida) showed varying levels of mortality, with coffee senna extract achieving the highest rate of 100%, followed by sesbania extract with 80% mortality. Storage duration of the extracts may have influenced their potency and changed the order of mortalities compared to previous results.

Objective 5: The impacts of sicklepod extracts on non-target beneficial bee pollinators.

In Year 3, the objective focused on evaluating the impact of sicklepod extracts on non-target beneficial bee pollinators. The experiments involved preparing sicklepod extract at specific concentrations and conducting bee feeding experiments to assess mortality. The results indicated that anthraquinone concentrations up to 550 ppm did not result in honey bee mortality after 14 days of feeding (Fig. 17). The subsequent experiments aimed to investigate the transportation of anthraquinone in soybean plants and the concentrations of anthraquinone in soybean flowers. Spraying soybean plants with sicklepod extract led to the transportation of anthraquinone in shoot tips and higher concentrations in treated plants compared to untreated plants (Fig. 18). However, the anthraquinone concentrations in treated plants rapidly decreased within a short period of 2.5 days, while untreated plants consistently exhibited lower concentrations. This raised questions about the distribution of anthraquinone in untreated flowers by unknown pollinators and their contributions to the compound's distribution. Further investigations are needed to identify these pollinators and gain a comprehensive understanding of the ecological significance and potential applications of anthraquinone in the context of bee pollination.

Overall, the findings from these objectives highlight the potential of sicklepod fractions and extracts for effective deer and insect management in soybean crops. The research demonstrates the importance of optimizing formulations, evaluating long-term effects, and considering the ecological impacts on beneficial pollinators to develop sustainable and effective pest management strategies.

IMPACTS AND BENEFITS TO MISSISSIPPI SOYBEAN PRODUCERS

The primary beneficiaries of the project are all soybean growers in Mississippi, who represent over 2.3 million acres across the state. The estimated average yield for soybean in Mississippi is about 46 bushels per acre, and the soybean production in 2016 was estimated at 112 million bushels or $900 million in production value. Considering up to 26% and 41% yield reduction caused by deer and insect herbivory, respectively, the estimated economic loss could be $234 and $369 million annually in Mississippi. Developing a low-cost and effective sprayable solution is the first step in solving this problem.

Indirect benefits: Applying the sicklepod extract via tank-mix with herbicides leads to significant savings in production costs and time. This also reduces the cost of labor incurred in spraying sicklepod extract and herbicides separately. The captive facility and field screening confirm sicklepod, coffee senna, and/or hemp sesbania extract formulations with the most effective anti-herbivore potential. Through this study, we also confirm whether our sicklepod extract adversely affects non-target beneficial bee pollinators. The availability of natural anti-herbivory compound formulations identified in this study leads to an increase in the environmental sustainability of agriculture with reductions in the need for synthetic pesticides.

Direct benefits: The project identifies anthraquinone formulations (developed using sicklepod, coffee senna, and/or hemp sesbania seeds) effective in repelling insects and deer (anti-herbivory). The other part of the project is to determine the specific anti-herbivory compounds associated with the anti-herbivory trait. These compounds can be used in identifying genes useful in molecular breeding to breed anti-herbivory traits into soybean. Soybean with a significant anti-herbivore property prevents yield losses incurred due to herbivores, especially deer and insects.

GRAPHICS/TABLES

Table 1. The effect of anthraquinone concentrations on soybean looper biomass. Sicklepod extract was fractionated by a C4 column, the fractions of the sicklepod extract were made into 2000 ppm dispersions in 0.5% crop oil. Soybean loopers at the 2nd instar stage were fed with soybean leaves treated with these dispersions for 5 days.

Fractions	**Looper biomass after 5 days (mg)**	**Anthraquinone concentrations (%)**
A	50	0
B	54	0
C	46	0
D	33	15.64
E	38	1.15

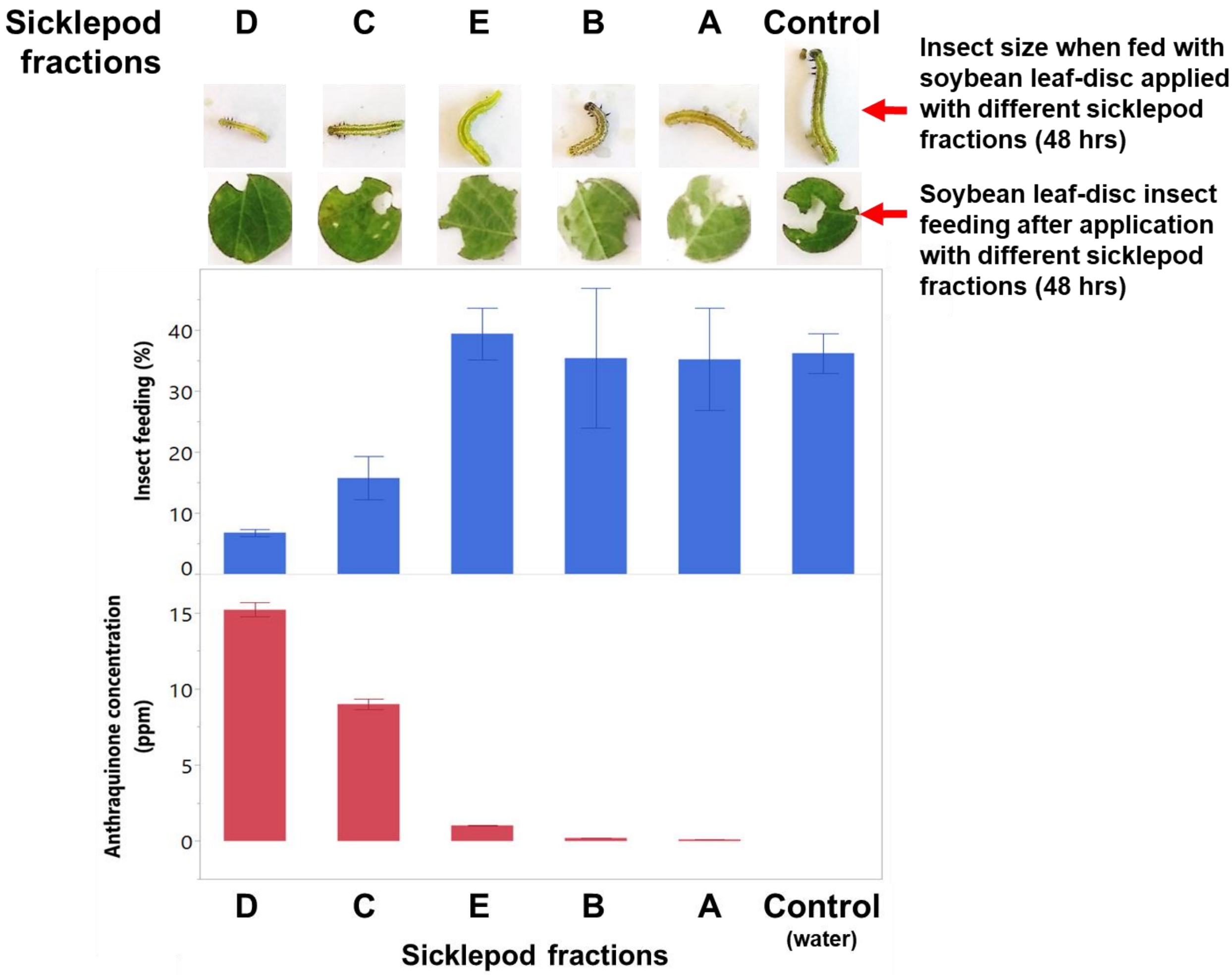

Figure 1. Anthraquinone content of different sicklepod fractions (A, B, C, D, and E) and its effect on insect feeding and insect size.

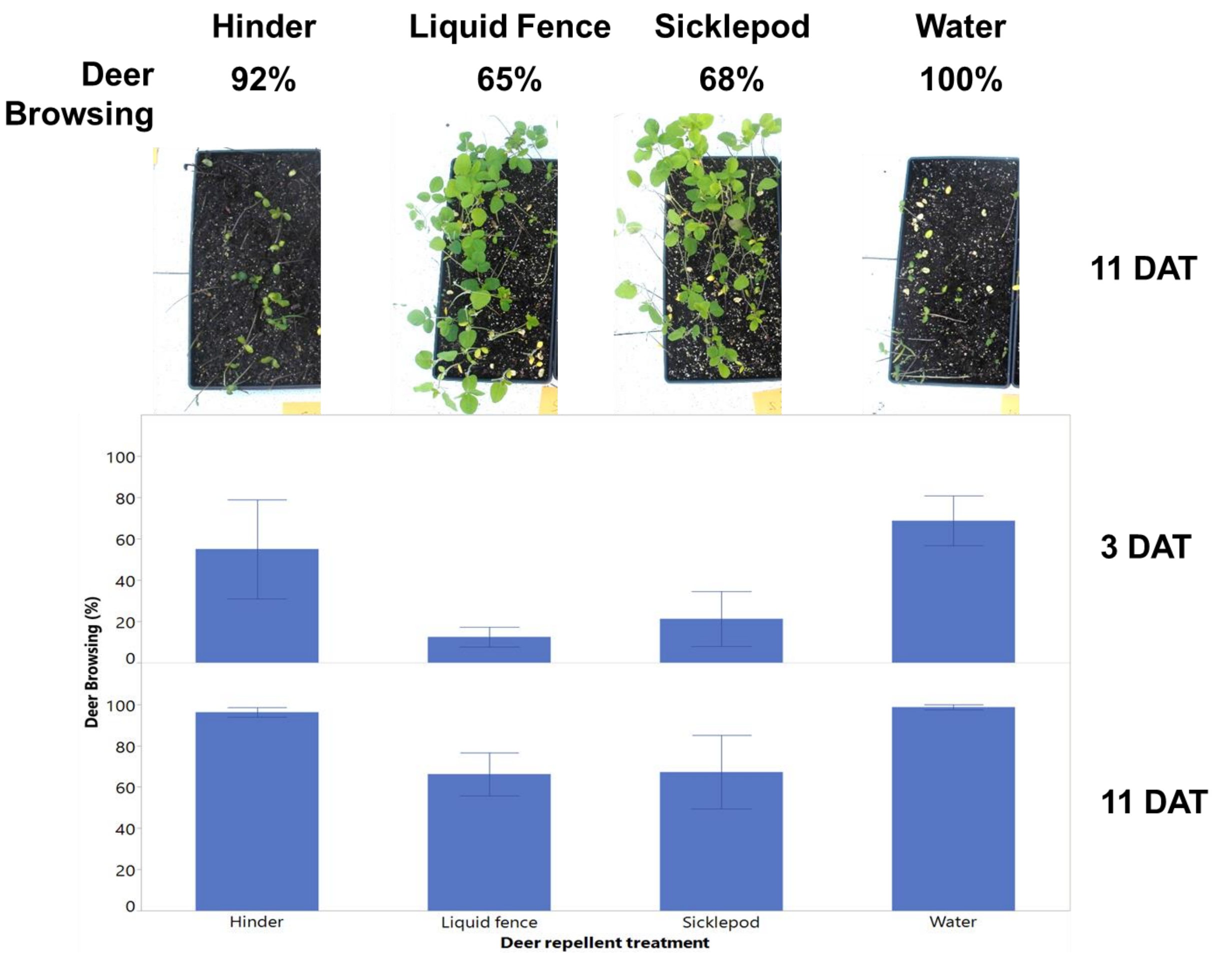

Figure 2. Soybean field trials showing deer browsing efficacy of sicklepod formulation compared to commercial deer (Hinder) and rabbit (Liquid Fence) repellents, and control (water).

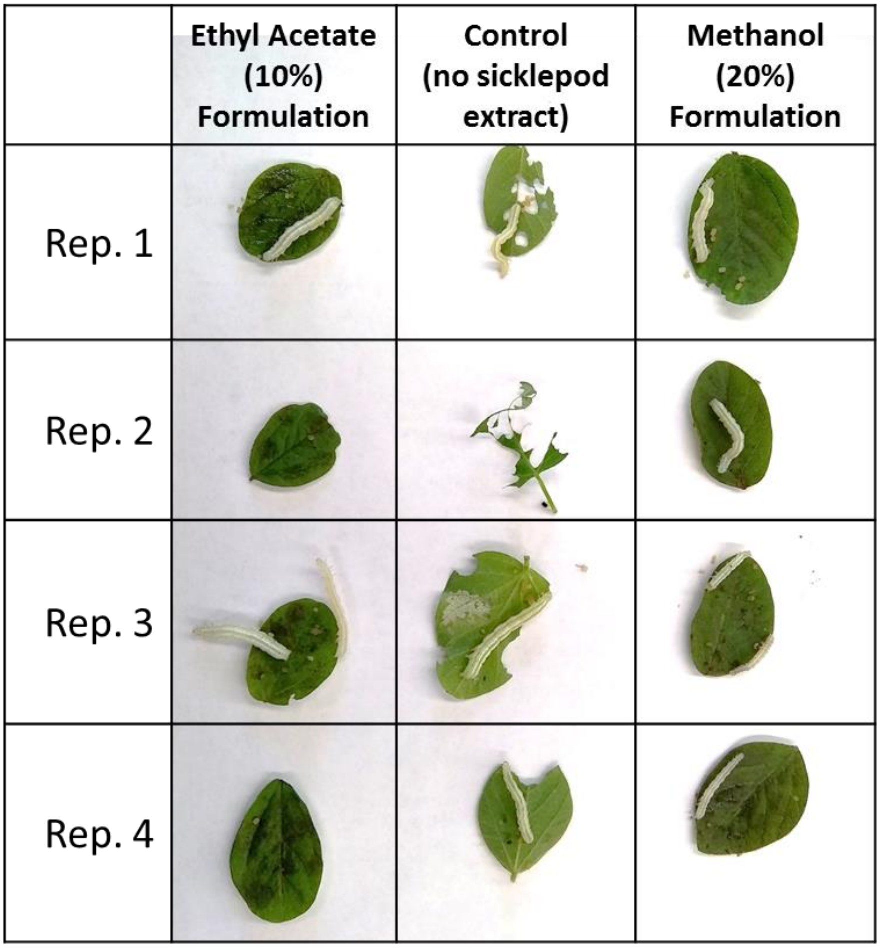

Figure 3. Soybean whole leaf assay to test different sicklepod extract formulations on soybean looper feeding. Both ethyl acetate and methanol formulations were effective in protecting soybean leaves from soybean looper feeding, compared to the control treatment where water was applied instead of sicklepod extract. The experiment was replicated four times and repeated twice. Two soybean loopers were added to each leaf. Not all insects were placed on the leaf when taking the above photo.

(A) browsing above cotyledon. (B) browsing below cotyledon

Figure 4. Soybean seedlings browsed by deer above (A) and below (B) the cotyledon leaves.

Figure 5. Field plot in 2021 showing a sicklepod treated row of soybean.

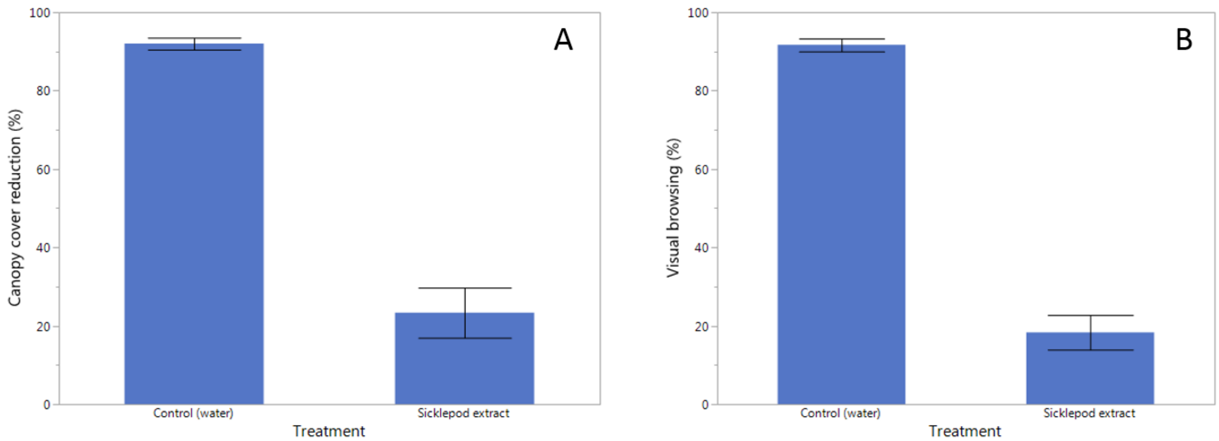

Figure 6. Canopy cover reduction (%) (a), and visual browsing (%) (b) of soybean seedlings treated with water (control), and sicklepod seed extract, after 4 hr of exposure to captive deer at the Captive Deer Facility at Mississippi State University. Images of soybean canopy were captured before and after deer exposure and analyzed using image analysis software Image J to determine percent canopy cover reduction caused by deer browsing. Visual browsing was recorded on a scale of 0 to 100% where 0 = no browsing, and 100 = completely browsed. Soybean plants applied with sicklepod extract showed 4 times greater protection from deer browsing than untreated soybean plants.

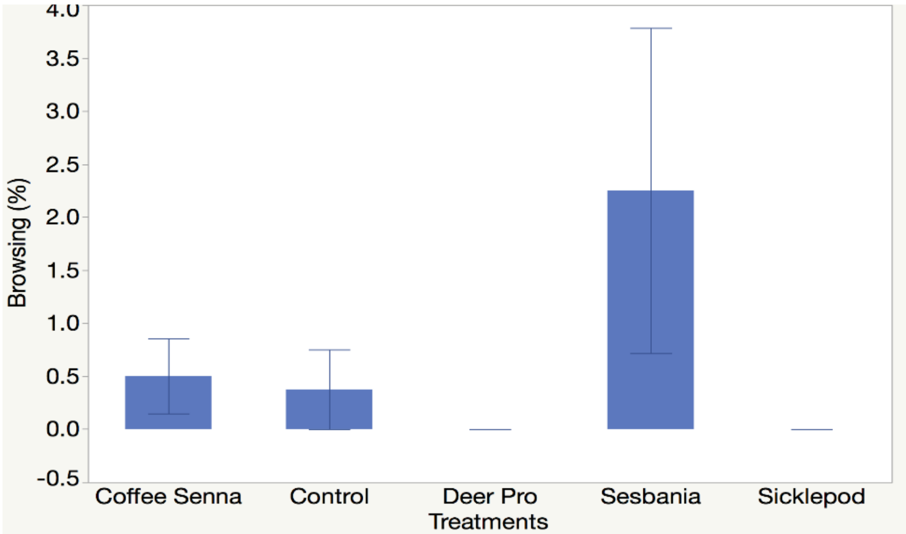

Figure 7. The effect of extracts of coffee senna, hemp sesbania, and sicklepod on soybean deer browsing. DeerPro, a commercial deer repellent, was included for comparison. Sicklepod extract, a natural plant extract, was as effective as DeerPro. The deer browsing was generally low because the study was conducted in late November. The study will be repeated in spring 2022.

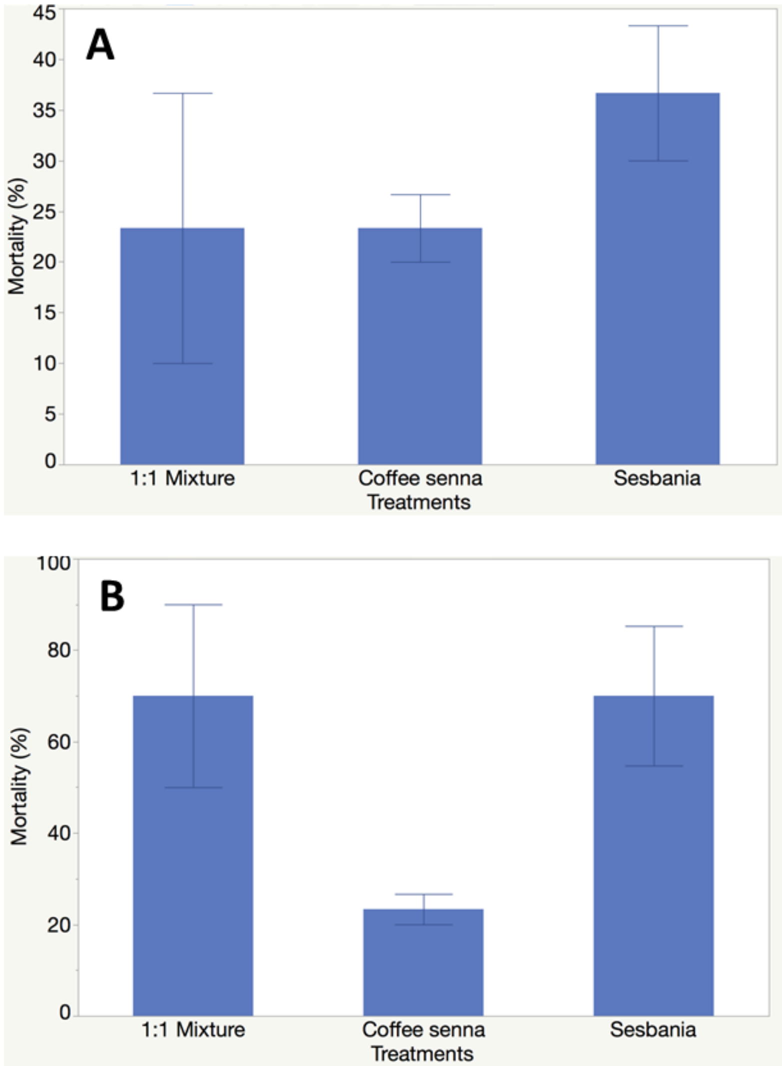

Figure 8. Mortality of soybean loopers at (A) 2nd instar (young larvae) and (B) 4th instar (adult) at 24 hours after exposure with the leaves treated with coffee senna, hemp sesbania, and mixture extracts.

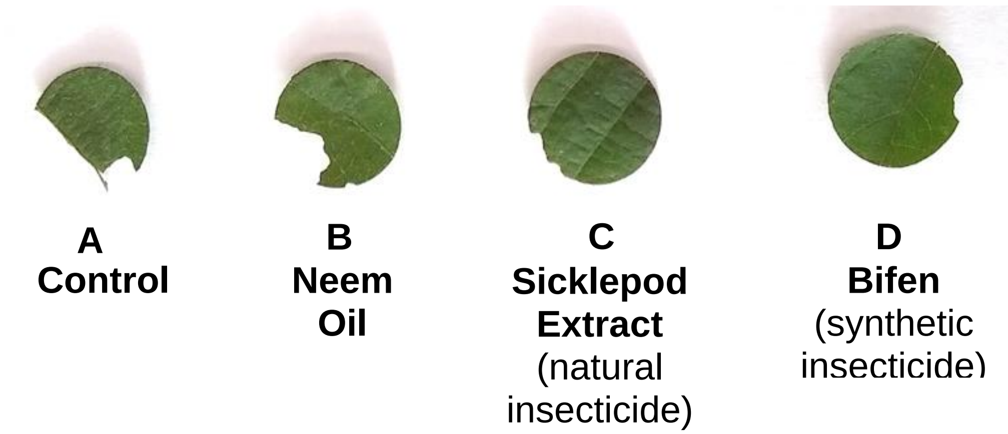

Figure 9. Soybean leaf disc images after 48 hour-feeding with two loppers per cup and supplemented with an alternate food source. The treatments from left to right are: A, control; B, neem oil; C, sicklepod extract; and D, bifen.

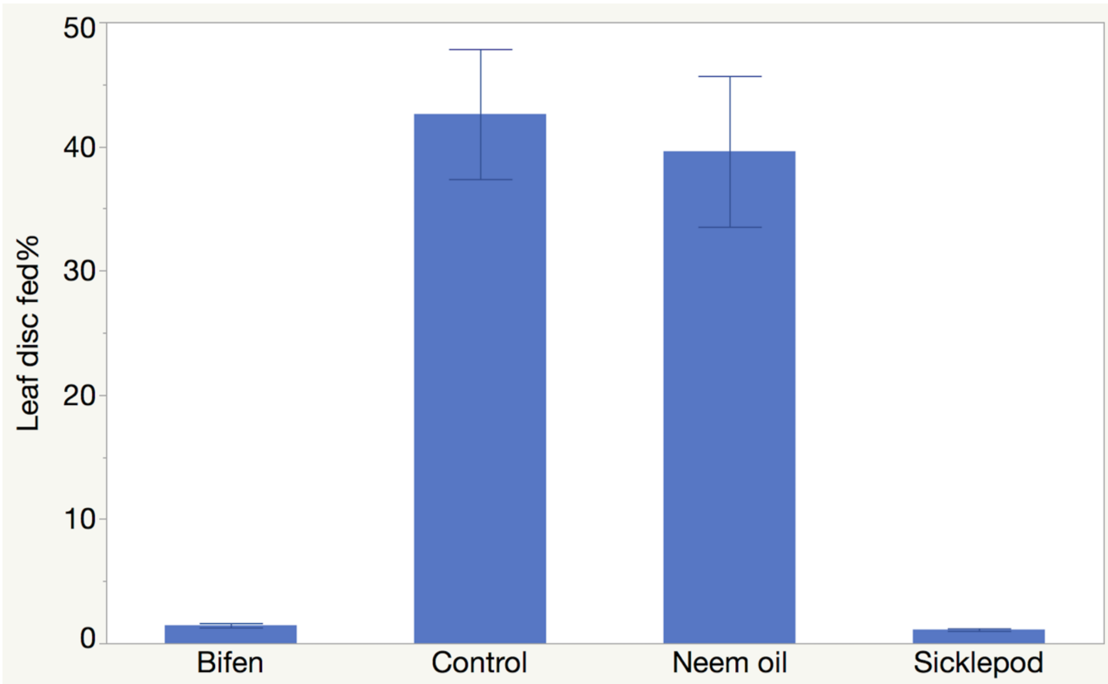

Figure 10. Soybean leaf percentage fed after 48 hours. The statistical letters indicate sicklepod extract and bifen had a similar antifeedant effect, while neem oil and control had same and higher insect feeding (n = 10).

Figure 11. Starkville soybean deer plots (R3 stage). The soybean was planted on May 9, 2022.

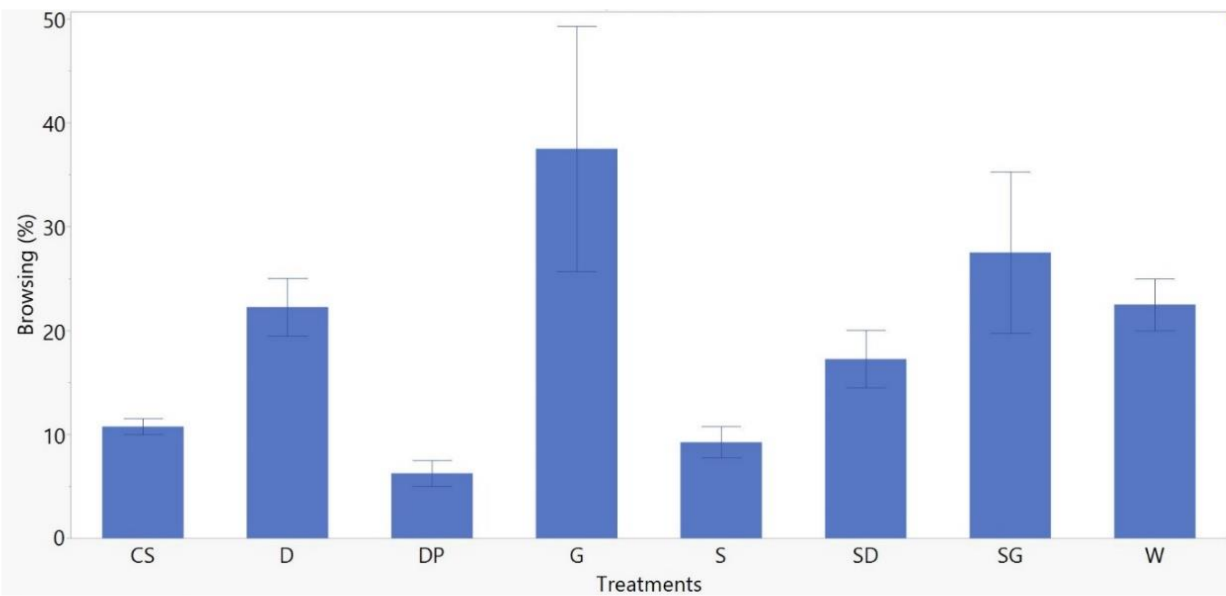

Figure 12. Deer browsing at Pontotoc site 12 weeks after treatment. CS, coffee senna extract; D, dicamba; DP, Deer Pro; G, glyphosate; S, sicklepod extract; SD, sicklepod extract + dicamba; SG, sicklepod extract + glyphosate; W, water control.

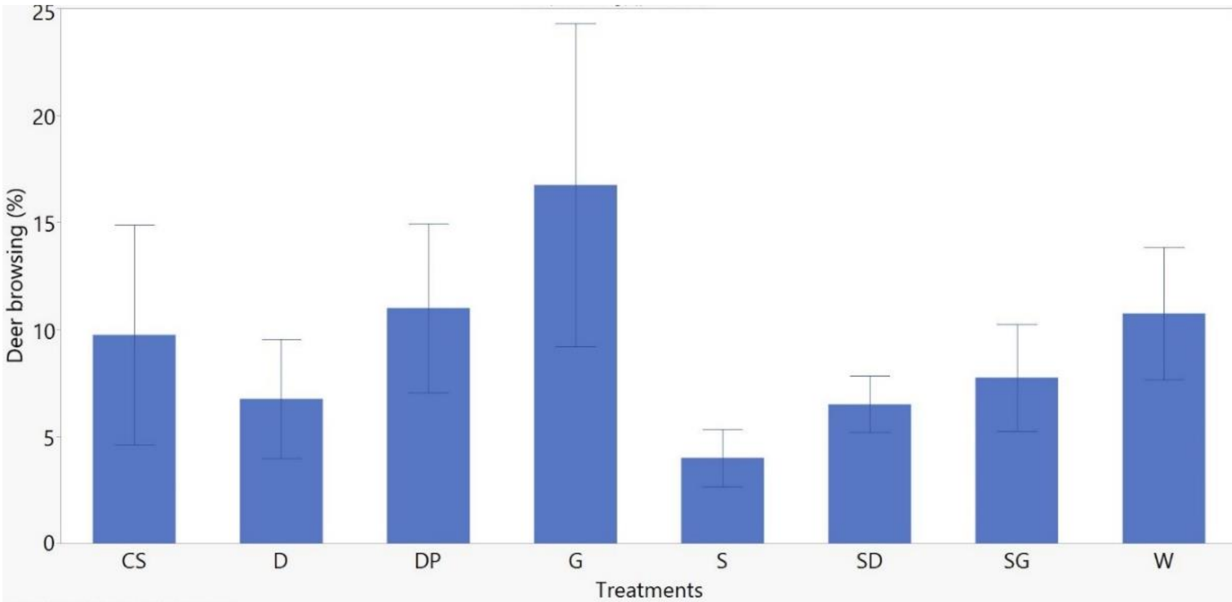

Figure 13. Deer browsing at Starkville site 12 weeks after treatment. CS, coffee senna extract; D, dicamba; DP, Deer Pro; G, glyphosate; S, sicklepod extract; SD, sicklepod extract + dicamba; SG, sicklepod extract + glyphosate; W, water control.

Velvetbean caterpillar (VBC) Green cloverworm (GCW) Soybean looper (SL)

Figure 14. Major insect pests present in the soybean plots. Insect data were collected using shake cloth with the following identification criteria: soybean looper (SL): two hind legs; green cloverworm (GCW): three hind legs; velvetbean caterpillar (VBC): four hind legs.

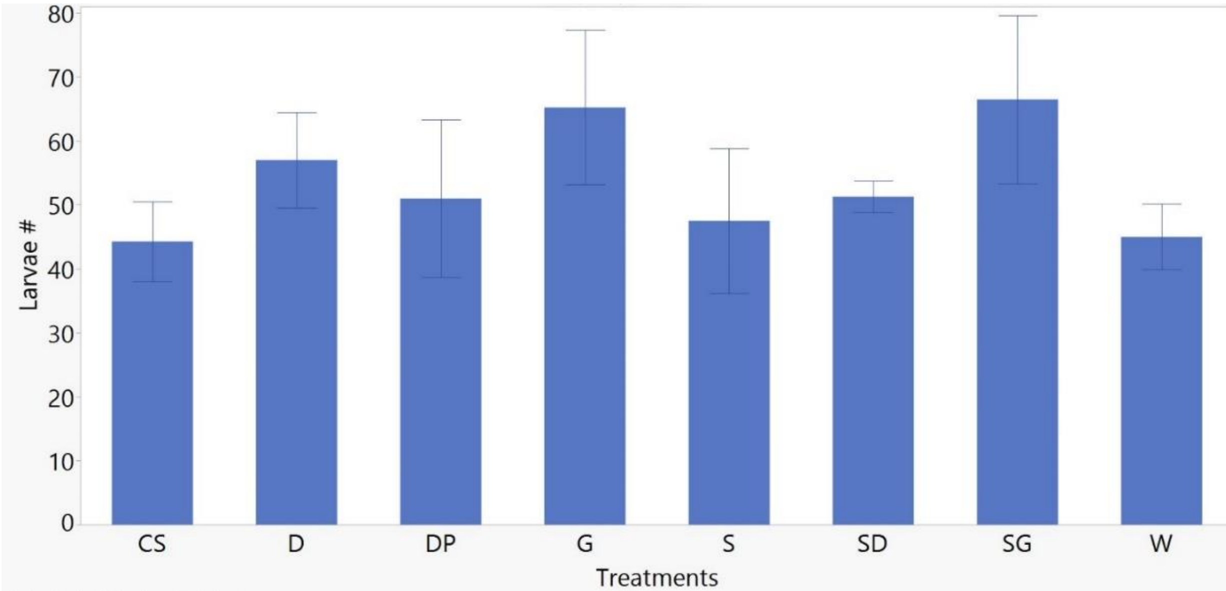

Figure 15. Starkville larvae number across treatments. Larvae no. = GCW no. + SL no. + VBC no. VBC, velvetbean caterpillar; GCW, green cloverworm; SL, soybean looper; CS, coffee senna extract; D, dicamba; DP, Deer Pro; G, glyphosate; S, sicklepod extract; SD, sicklepod extract + dicamba; SG, sicklepod extract + glyphosate; W, water control.

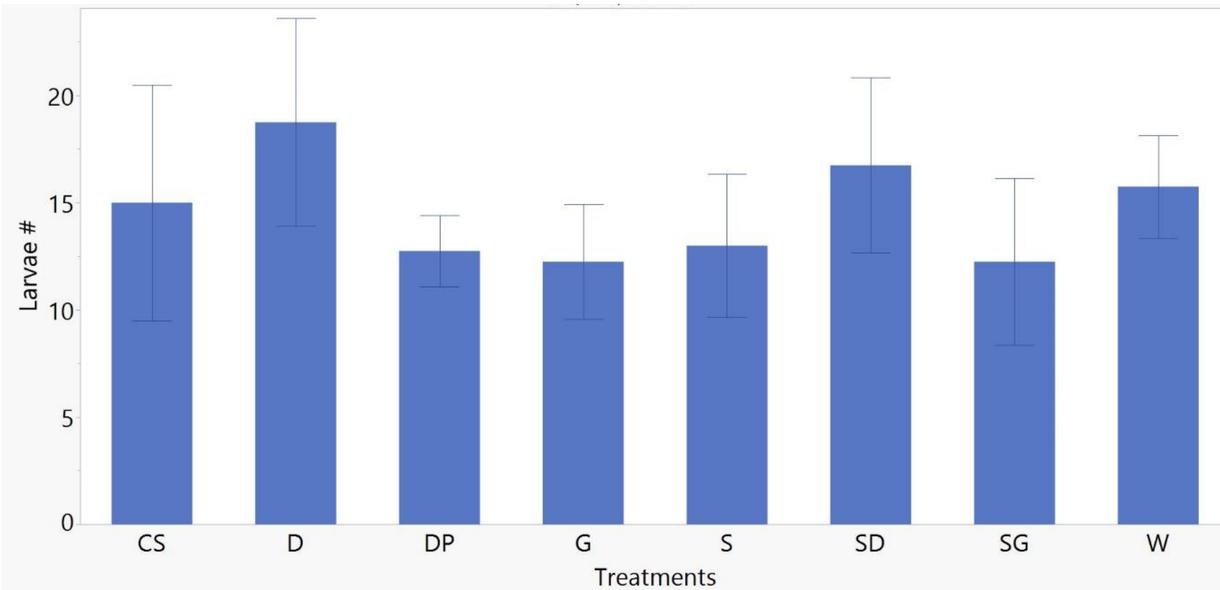

Figure 16. Pontotoc larvae number across treatments. Larvae no. = GCW no. + SL no. + VBC no. VBC, velvetbean caterpillar; GCW, green cloverworm; SL, soybean looper; CS, coffee senna extract; D, dicamba; DP, Deer Pro; G, glyphosate; S, sicklepod extract; SD, sicklepod extract + dicamba; SG, sicklepod extract + glyphosate; W, water control.

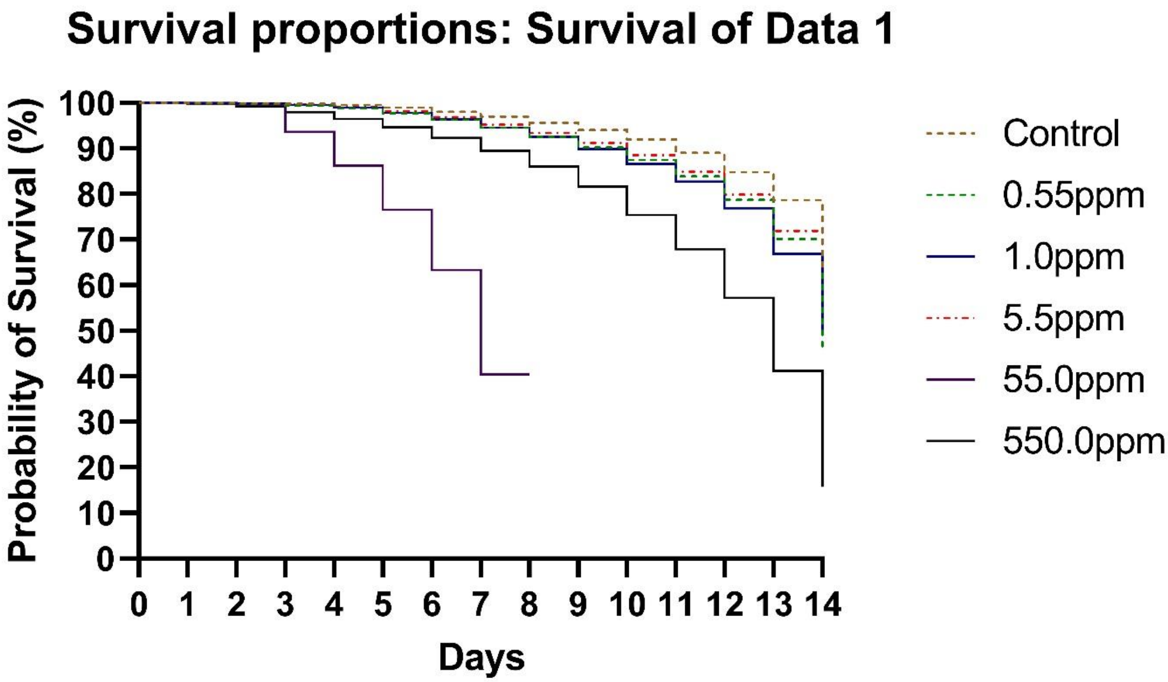

Figure 17. Probability of survival of honey bees fed with anthraquinone concentrations ranging from 0 (control) to 550 ppm.

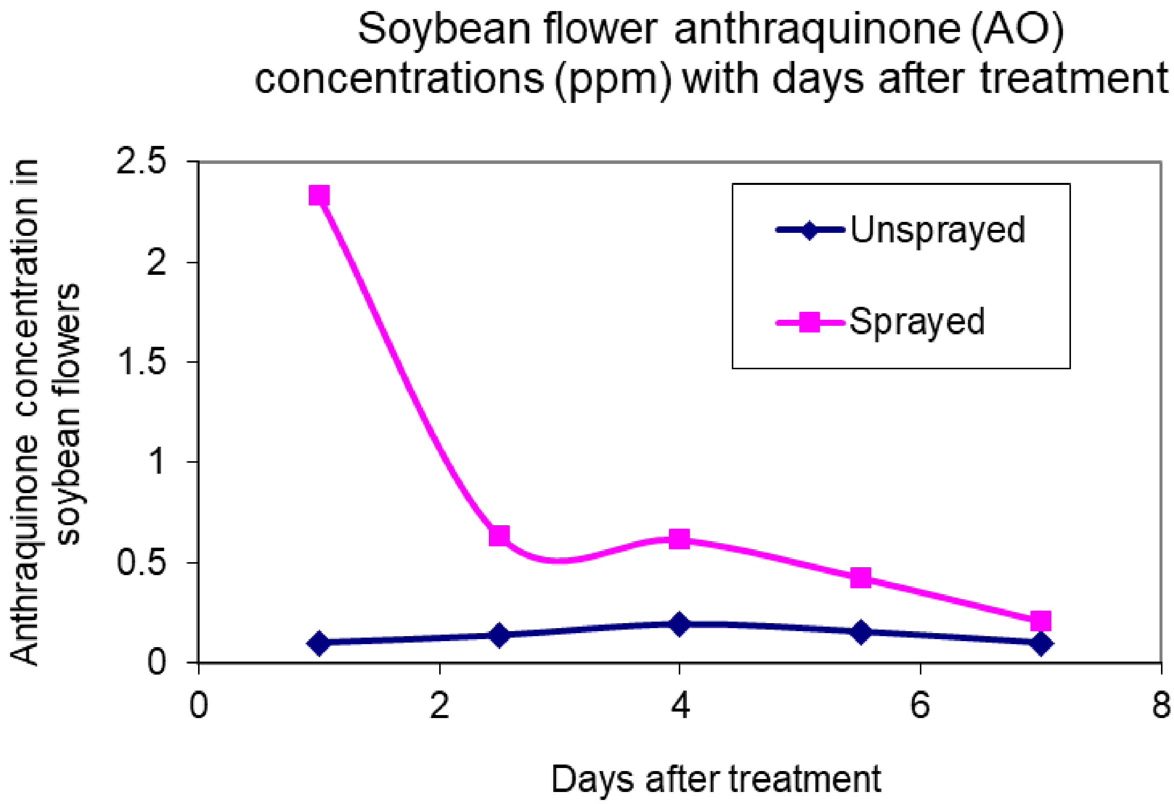

Figure 18. Anthraquinone concentrations (ppm) in soybean flowers at different days after treatment with sicklepod extract.