06-2021, Sicklepod extract formulations as natural and effective deer and insect repellent

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RATIONALE/JUSTIFICATION FOR RESEARCH: 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 previously funded MSPB project, 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 (**Fig. 2**). Moreover, the sicklepod extract had no adverse effect on the overall soybean yield (**Fig. 3**). Our preliminary leaf disc insect feeding results indicated 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 (**Fig. 4**). The antifeedant effect of sicklepod extract and Bifen were similar, while the control and neem oil treatments had lower and similar antifeedant effects (**Fig. 4**). In Year 1, we prepared and characterized four sicklepod fractions (A, B, C, D, and E) with improved deer and insect repelling efficacy. Leaf disc assays showed that fraction D and C resulted in 80 and 52% reduction in soybean looper feeding (**Fig. 5**). Insects fed with soybean leaves treated with fraction D and C were also the smallest in size (**Fig. 5**). Additionally, in our chromatography (HPLC) analysis, fractions D and C showed the highest anthaquinone concentrations (15 and 8 ppm, respectively), thus confirming the insecticidal property of anthaquinone. Soybean field trials conducted in Year 1 showed that our sicklepod formulation was most effective in repelling deer; better than Hinder (commercial deer repellent) and control (water) (**Fig. 6**).

The limit our current sicklepod extract formulation is the high viscosity, which prevents the formulation of an extract with high anthraquinone concentration (ideally >300 ppm)—increasing the anthraquinone concentration of the extract results in increased viscosity of the spray fluid, making it hard to apply through spray booms. Sicklepod seeds contain large amounts of galactomannans; a polysaccharide often used to increase the viscosity of solutions, primarily upon heating. There is, therefore, a critical need to increase the efficacy of the sicklepod extract by increasing its active ingredient concentration (anthraquinone concentration to >300 ppm; at present only <100 ppm) and decrease the viscosity (not to clog spray). Our preliminary experiments show sicklepod seeds extracted in methanol (20%) or ethyl acetate (10%) resulted in high anthraquinone concentrations (>200 ppm), resulting in effective leaf antifeedant effect against soybean loopers (**Fig. 7**). In addition, a nonionic surfactant can be used to increase spreading of the extract on soybean leaf surface to provide long-lasting insecticidal and deer-repelling effects. The use of organic solvents such as methanol, chloroform, acetone, for extracting anthraquinone in sicklepod will allow the removal of the galactomannans, eventually increasing the concentration of anthraquinone to >300 ppm in the extract.

OBJECTIVES:

(1) repeat the captive deer trial to determine the most effective formulation (from objective #1) in preventing soybean browsing;
(2) repeat the soybean leaf disc assay to determine the most effective formulation (from objective #1) for preventing insect feeding in soybean; and,
(3) use effective formulations (from objective #2) to repeat field trials in forestry and agronomy research fields using unmanned aerial vehicles (UAVs), plant surveys, and trail cameras to quantify deer and insect use and damage to soybeans in treatment and control plantings.

REPORT OF PROGRESS/ACTIVITY:

- Studies conducted by us in 2020 showed that soybean yields between glyphosate and glyphosate+sicklepod extract treatments were similar, indicating that sicklepod extract tank-mixed with glyphosate does not adversely affect soybean yield. Through the 2022 project we plan to confirm if the sicklepod-herbicide (glyphosate and dicamba) tank-mix does not affect weed, deer, and insect control.
- High-performance liquid chromatography analysis of coffee senna (closely related to sicklepod) extract showed a higher amount of anthraquinone derivative than siklepod. Coffee senna also has lesser concentration of galactomannose (therefore, less viscous). Combining sicklepod and coffee senna in an appropriate ratio may thus allow us to increase the anthraquinone concentration of the extract formulation without increasing the viscosity resulting in the ease of application.
- Deer repellent:
  - Soybean plants applied with sicklepod extract showed 4 times greater protection from deer browsing than untreated soybean plants (**Fig. 1**). This findings is based on the canopy cover and visual browsing of soybean seedlings, after 4 hr of exposure to captive deer at the Captive Deer Facility at Mississippi State University.
  - Sicklepod extract, a natural plant extract, was as effective as DeerPro in protecting soybean plants from deer browsing (**Fig. 2**)
- Insect repellent:
  - 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 (**Fig. 3, 4**). The antifeedant effect of sicklepod extract and Bifen were similar, while the control and neem oil treatments had lower and similar antifeedant effects.
  - Coffee senna and hemp sesbania extracts also lead to up to 35 and 70% mortality of soybean loopers at the 2nd and 4th instar, 24 hours after exposure to soybean leaves treated with coffee senna, hemp sesbania, and mixture extracts (**Fig. 5**).
  - Comparison between anthraquinone concentrations and looper biomass leads us to conclude anthraquinone derivatives are the active ingredients of the sicklepod extract as a looper antifeedant (**Table 1**).

IMPACTS AND BENEFITS TO MISSISSIPPI SOYBEAN PRODUCERS

The weed formulations were more effective than the commercial products, Hinder and DeerPro (deer repellent), and Bifen (insecticide). The availability of natural and effective anti-herbivory compound formulations identified in this study will potect soybean yield from white-tailed deer herbivory resulting in a profit of up to $68/ha over one growing season (Conover 2002). The adoption of these forumlations will also lead to an increase in the environmental sustainability of agriculture with reductions in the need for synthetic pesticides.

END PRODUCTS – COMPLETED OR FORTHCOMING
Publications (2021-2022)

1. Yue Z., C. L. Cantrell, N. Krishnan, D. J. Lang, M. Shankle, T. M. Tseng (2021) Characterization of sicklepod extract as a deer repellent and insecticide for soybean looper (Lepidoptera: Noctuidae). In *Proceedings of Weed Science Society of America*, vol. 61, p. 263. Oral
2. Yue Z., C. L. Cantrell, N. Krishnan, D. J. Lang, M. Shankle, T. M. Tseng (2021) Characterization of sicklepod extract as a deer repellent and insecticide for soybean looper (Lepidoptera: Noctuidae). In *Proceedings of Southern Weed Science Society*, vol. 74, p. 84. Oral
3. Z. Yue*, N. Krishnan, T.M. Tseng (2022) Efficacy of Sicklepod Extract for Protection of Soybean from Soybean Loopers (*Chrysodeixis includens* (Walker)). In *Proceedings of Southern Weed Science Society*, vol. 75, p. 113. Poster
4. Z. Yue, M. W. Shankle, P. S. Sharma, A. L. Miller, T. M. Tseng (2022) Efficacy of Plant Based Sicklepod Deer Repellent in Soybean Production System. In *Proceedings of Southern Weed Science Society*, vol. 75, p. 126. Oral

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

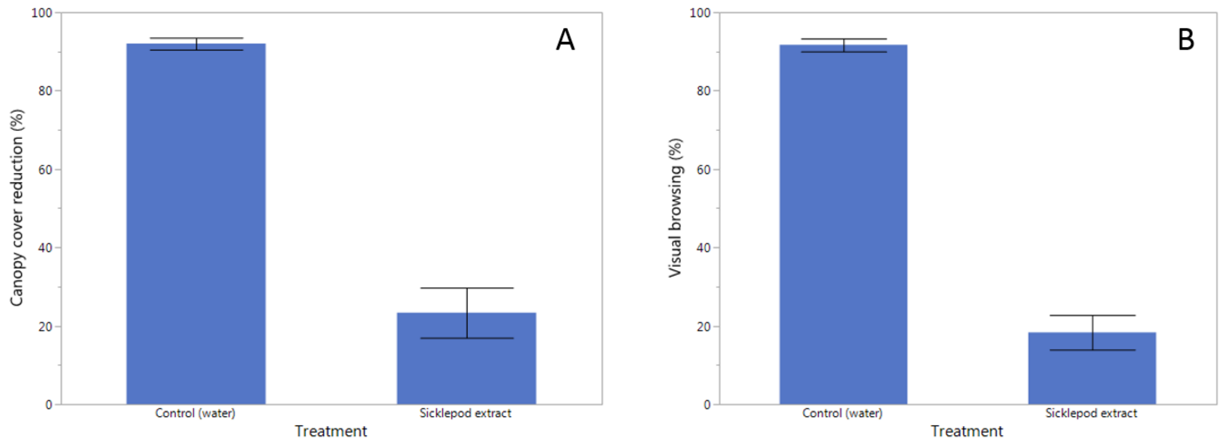

Figure 1. Canopy cover reduction (%) (a), and visual browsing (%) (b) of soybean seedlings treated with water (control), and sicklepod seed extract, after 4 hr of exposure to captive deer at the Captive Deer Facility at Mississippi State University. Images of soybean canopy were captured before and after 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.

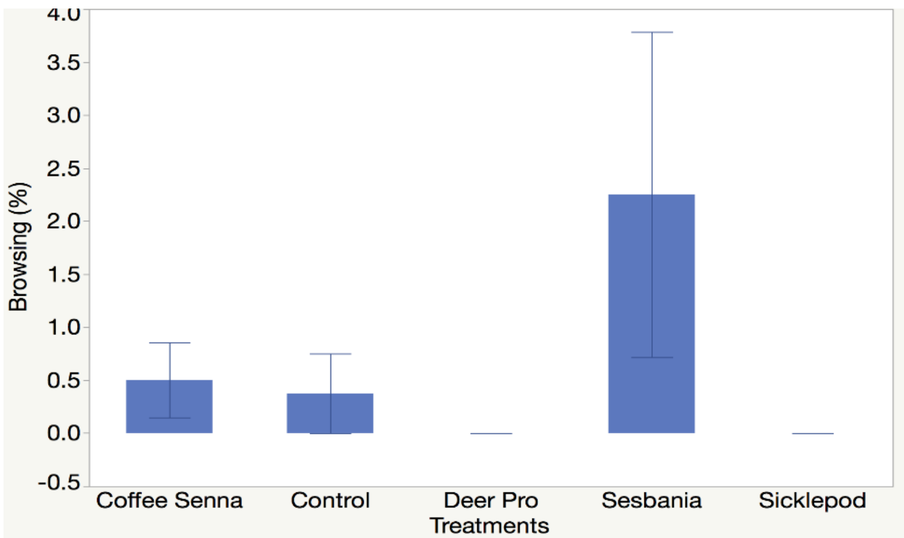

Figure 2. The effect of extracts of coffee senna, hemp sesbania, and sicklepod on soybean deer browsing. DeerPro, a commercial deer repellent, was included for comparison. Sicklepod extract, a natural plant extract, was as effective as DeerPro. The deer browsing was generally low because the study was conducted in late November. The study will be repeated in spring 2022.

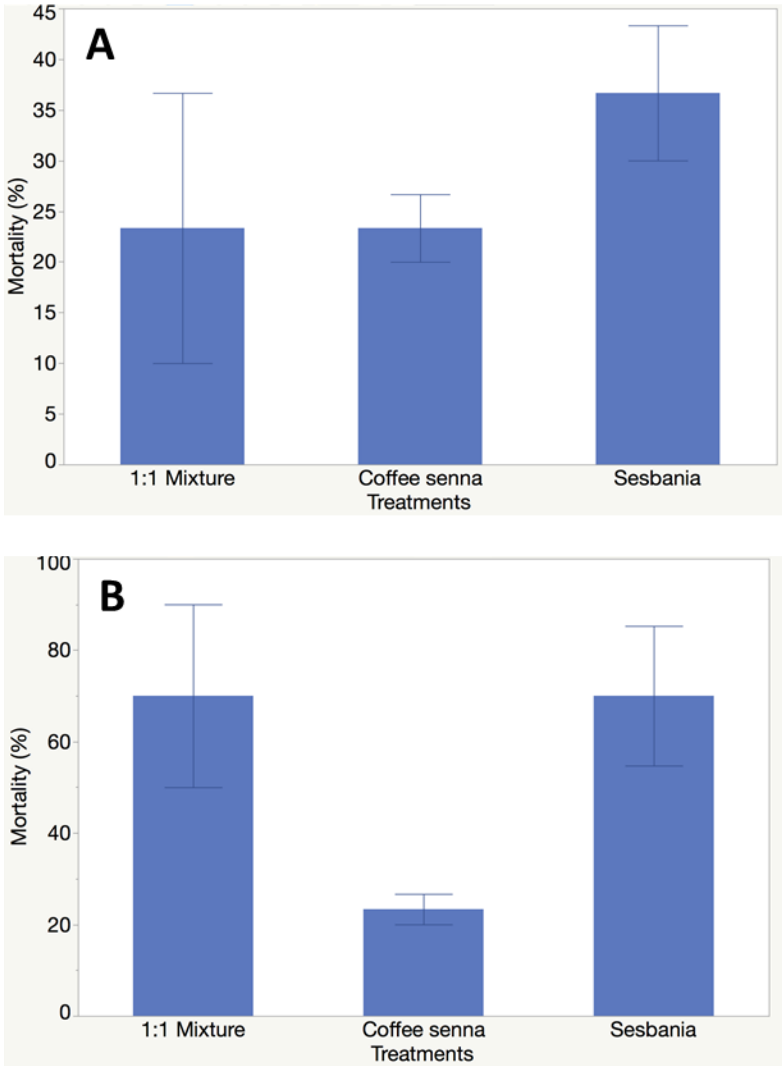

Figure 3. Mortality of soybean loopers at (A) 2nd instar (young larvae) and (B) 4th instar (adult) at 24 hours after exposure with the leaves treated with coffee senna, hemp sesbania, and mixture extracts.

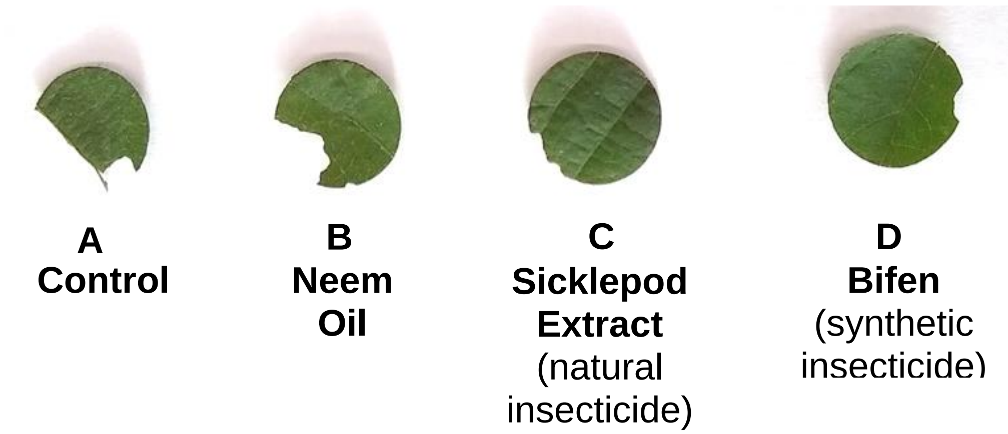

Figure 4. Soybean leaf disc images after 48 hour-feeding with two loppers per cup and supplemented with an alternate food source. The treatments from left to right are: A, control; B, neem oil; C, sicklepod extract; and D, bifen.

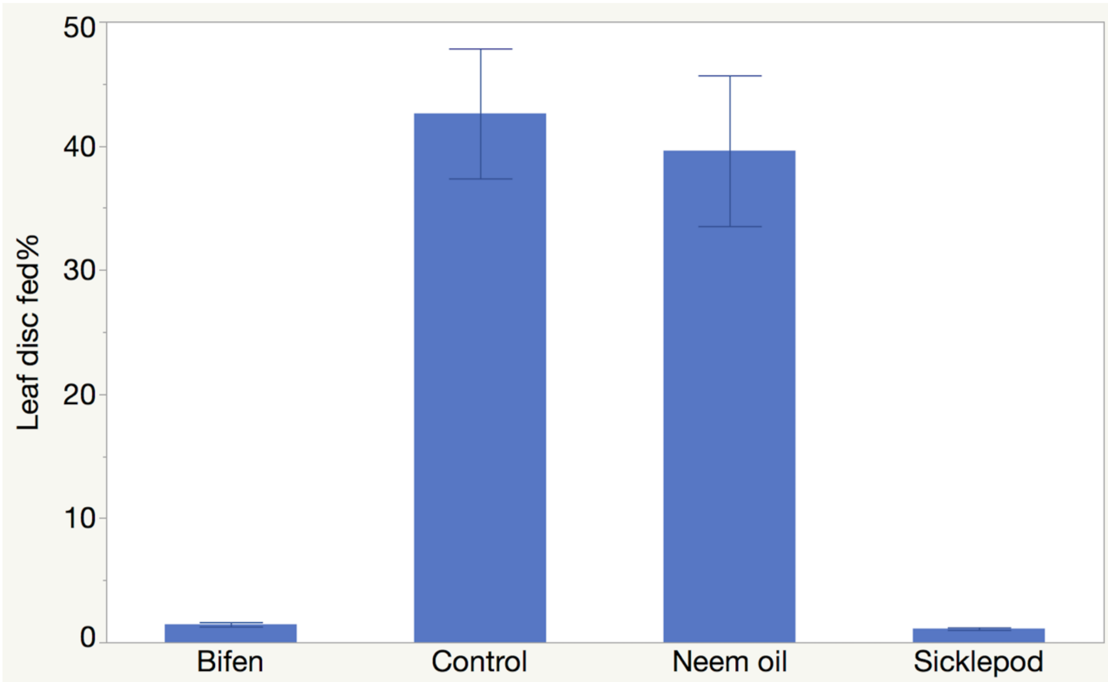

Figure 5. Soybean leaf percentage fed after 48 hours. The statistical letters indicate sicklepod extract and bifen had a similar antifeedant effect, while neem oil and control had same and higher insect feeding (n = 10).