*International Journal of Organic agricultural Research & Development*
*Volume 19 (2024)*

# CHEMICAL CHARACTERIZATION OF *Hyptis suaveolens* AND *Bridelia ferruginea* AND THEIR ASSESSMENT FOR NEMATICIDAL PROPERTIES AGAINST *Meloidogyne incognita* INFECTING SOYBEAN (*Glycine max)*

**Nkechi Betsy Izuogu, Aishat Adetola Anifowose*, and Azeezat Bukola Adigun**
Department of Crop Protection, Faculty of Agriculture, University of Ilorin, P.M.B. 1515, Ilorin, KW 240102, Nigeria
*Correspondence: anifowose.aa@unilorin.edu.ng

## ABSTRACT

Soybean production is constrained by root-knot nematodes (RKNs) that attack the root system leading to reduced productivity in Nigeria. Thus, these field and screenhouse experiments investigated the use of powdered leaf extracts of *Hyptis suaveolens and Bridelia ferruginea* as plant-based nematicides in the control of RKNs infecting two varieties of soybean.The studies were 2 x 3 factorial experiments fitted into a randomized complete block design,. Each pot in the screenhouse was inoculated with 1000 eggs at planting, whereas the field was naturally infested. *Hyptis suaveolens and Bridelia ferruginea* were applied at 200 g per stand, twice, at a four-week interval, first at one week after planting. The untreated pots and plots served as the controls for the experiments. Phytochemical screening was conducted on samples of the plant materials to determine the presence of organic compounds. Data collected on growth parameters, pod production, and nematode population were subjected to analysis of variance and means were separated using the Fisher's Least Significance Difference Test at P=0.5. The results revealed that the treated plants recorded significantly higher (P=0.5) growth and yield than the controls. The untreated TGXP1448-2F plants recorded the highest final nematode populations. In spite of the susceptibility of both varieties to *M. incognita*, powdered form of *Hyptis suaveolens* had significantly higher performance than that of *Bridelia ferruginea*, especially on the LOCAL variety. Phytochemical screening of the botanicals revealed the presence of certain organic compounds including phenol and phytol. The prospects of combined powdered extracts of *Bridelia ferruginea* and *Hyptis suaveolens* as efficacious integrated nematode management strategy in soybean production is intriguing.

**Keywords**: Soybean, *Meloidogyne incognita,* organic nematicides*, Bridelia ferruginea, Hyptis suaveolens,* Phytochemical screening

## INTRODUCTION

Soybean (*Glycine max* (L.) Merr.) is an industrial crop supplying approximately half of the world demand for vegetable oil (Nieto and Lorenzo, 2022). It has the highest protein quality among plant proteins and the protein quality is comparable to some animal proteins. Given the risks associated with excessive intake of animal proteins like red meat, soybean can serve as substitute for animal products because, it contains essential amino acids and less fat content unlike other plant proteins hence no major dietary adjustment is needed (Ahnen *et al*., 2019). Besides its use in livestock feed and industrial purposes, it lowers blood pressure, improves blood vessels, improves bone health, protects against breast cancer, improves cognitive function and visual memory (Rizzo *et al*., 2018). For these reasons, its global consumption and production is increasing. However, there is a huge gap in the yield of the crop in Nigeria (1,049,000 T) compared to Brazil (122 MT), USA (113 MT), etc. (FAOSTAT, 2019), yet Nigeria is the second-largest producer of the crop in Africa. This is among other factors, due to the crop's susceptibility to pests and diseases including the cosmopolitan nematodes especially the root-knot nematode that parasitizes its root systems. Chemical nematicides are not only expensive and out of reach for the rural-dwelling farmers, but also poses several serious health and environmental concerns. Thus, the need for cost effective and environment-friendly alternative control measures.

Many plants have been tested and confirmed with varying efficacies, as intriguing phytochemical compounds either as plant extracts or soil amendments, (Prakash and Rao, 2018).

Botanical pesticides are biodegradable, renewable, remain active across various agro-climatic zones, seasons and crops; maximize crop yields, protect the environment, biodiversity and human health; and exhibit selective toxicity to target pests (Neeraj et al., 2017). The pesticidal abilities of extracts from several plant parts including roots, tree barks, leaves, seeds and fruits have been tested on various economically-important plant-parasitic nematodes (PPNs) in crop production (Mokrini *et al*., 2018).

*Hyptis suaveolens,* like some other local plants have been reported to exhibit pesticidal activity in certain crops infected by nematodes. Okechalu *et al*. (2020) reported its nemastatic ability in *Meloidogyne incognita-infected* onions. There are various reports on the chemical composition of *Hyptis suaveolens* (Stevenson *et al*., 2017; Oscar *et al.* 2020) and how these components could possibly contribute to its antimicrobial functions. Few studies have thus incorporated *Hyptis suaveolens* in the preparation of environment-friendly nematicides with different results. Anifowose *et al.* (2023) tested and confirmed that incorporating leaves of *Hyptis suaveolens* in the preparation of effective microorganisms (EM); microbial inoculants that modifies treated soils to become disease suppressive resulted in a EM mix with interesting nematicidal activity. Such microbiota were made possible by compounds present in the EM mix (antioxidants), which are toxic to eggs and/or juveniles of nematodes (Kankam *et al*. 2015). Furthermore, combining the EM mix with compost manure provided even more tolerance to *Meloidogyne incognita* in sweet potatoes and tomato plants treated with the EM and compost manure mix (Anifowose *et al.* 2023; Sossou *et al.* 2022).

The study hence determined response of two varieties of soybean infested with root knot nematode to two plant based extracts.

## MATERIALS AND METHODS

### Experimental Sites

The field experiment was conducted at the Teaching and Research Farm, University of Ilorin, (8°29' N and 4°35' E) situated within the Southern Guinea Savannah ecological zone, while the pot experiment was carried out in the Screenhouse of the Department of Crop Protection, University of Ilorin between July and October 2018.

### Source of seed and inoculum

Seeds of two varieties of soybean: TGX1448-2F and LOCAL were obtained from a certified agro-allied company in the Ilorin metropolis while the galled roots of *Meloidogyne incognita* infested *Celosia argentea* plants were obtained from the Oyun area, within the Ilorin metropolis, Kwara State.

### Preparation of treatments

Leaves of *Hyptis suaveolens* and *Bridelia ferruginea* were obtained from around the University of Ilorin. The leaves were shredded, air dried and and blended into powdered form.

### Phytochemical Screening

Samples of the powdered leaves of *Hyptis suaveolens* and *Bridelia ferruginea* were subjected to gas chromatography-mass spectrometry analysis at the Department of Chemical Engineering Laboratory, Faculty of Engineering, University of Ilorin, to determine the bioactive compounds and essential oils present in *Bridelia ferruginea* and *Hyptis suaveolens*

Flushing and running of the samples was carefully done to ensure effective separation of the different constituents of the samples, and the identification of the various bioactive compounds:

### Flushing

The extraction solvent, in this case, methanol was used to flush the instrument to remove dirt and achieve uniform results. Three (3) wash bottles were placed in the GC column, two of which contained methanol while the last one was left empty. The syringe took solvent from the first and the second washed bottles three times each into the inner part of the GC and the injector, flushed the solvent and dispose the whole content into the empty bottle provided. This flushing was done repeatedly for 30 minutes (Sarker and Nahar, 2012).

### Running of the samples

The running of the sample took place after flushing. The rule is to not use more than 1uml of the extract so that the toxicity will not affect the machine's effectiveness.

Vial bottles containing the 1uml of the extracts were placed in the GC column (The GC used could run 15 samples at a time). The auto injector drew the extract up from the vial bottle and inject into the GC over a set temperature range for the machine (ion source temperature, 250$^0$C, interface temperature, 300$^0$C). Running of the samples took at least forty-five minutes. Ms Solution software provided by supplier was used to control the system and to acquire the data: Identification of the bioactive compounds and essential oils was carried out by comparing the mass spectra obtained with those of the standard mass spectra obtained from National Institute of Standards and Technology, NIST standard database library software (Mallard and Linstrom, 2008).

**Field experiment:**

**Experimental design**

The experimental field was ploughed, harrowed and ridged. It was designed as a 2x3 factorial experiment fitted into a randomized complete block design (RCBD) replicated four time. The first factor was Soybean variety at two levels: TGX1448-2F and LOCAL, and the second factor was the Plant extract source (*B. ferruginea, H. suaveolens* and Control).

The experimental field was divided into four blocks which comprised of 6 plots each, each plot measured 10 m x 3 m with alley of one metre. The field experimental plots were naturally infested, but the initial nematode population per 1kg of soil was 80 juveniles

**Screenhouse experiments**

The experimental design for the screenhouse trial was also a 2 x 3 factorial experiment fitted into a randomized complete block design, and there were five replicates. A total of thirty buckets perforated at the bottom were filled with sterilized soil and placed on elevated platform to avoid reinstestation. The first factor was Soybean variety at two levels: TGX1448-2F and LOCAL, and the second factor was the Plant extract source (*B. ferruginea, H. suaveolens* and Control).

**Extraction of eggs and juveniles from galled roots**

Galled roots of *Celosia argentea* plants was the source of inoculum. Nematode extraction was carried out at the Department of Crop Protection Laboratory and confirmed at the International Institute of Tropical Agriculture (IITA), Ibadan. 10 grams of galled roots with *M. incognita* of *Celosia argentea* plants were washed and chopped into small pieces. The eggs were extracted from the excised galls as described by Hussey and Baker (1973), whereas the Baermann tray extraction tray extraction method was employed to extract the juveniles. Overall, 1000 eggs were obtained from 10 grams of the galled roots and each pot in the screenhouse was inoculated with 10 grams of galled roots.

**Treatment preparation**

*Powdered forms of Bridelia ferruginea* and *Hyptis suaveolens* were applied at 200g per stand twice: first at one week after planting and finally at four weeks after the first treatment application

**Cultural practices**

In addition to weeding was maintained on the field and in the screenhouse.

Scarecrow was another cultural practice maintained on the field to ward off birds

**Data collection**

Data were collected on initial and post harvest nematode counts, plant height, fruit (unshelled) and pod weight (shelled). All numerical data were subjected to analysis of variance and significant difference in means were separated using the Fisher's Least Significance Difference Test at P=0.5.

## RESULTS AND DISCUSSION

The effect of variety was not significantly different for all parameters except fruit weight and final nematode populations (Tables 1, 2, 7, 8, 13 and 14). Variety 2 (LOCAL) had significantly lower final nematode population than variety 1 (Tables 13 and 14) and significantly higher pod, fruit, root, and shoot weight than variety 1 (Tables 7 and 8). Plants treated with powdery forms of *Bridelia ferruginea* and *Hyptis suaveolens* had significantly higher (p = 0.5) values for plant height (Tables 3 and 4), fruit weight and pod weight (Tables 9 and 10) than the control plants. Meanwhile, the treatment effect was not significantly different for plant height between plants treated with powdery forms of either of the plant extracts (Tables 3 and 4). And treatment 2 recorded significantly lower final nematode populations than treatment 1 and control.

The effect of interaction between the treatment and variety was significant for all parameters (Tables 5, 6, 11, 12, 17, and 18). Treatment 2 with variety 2 performed significantly higher than with variety 1 and control plants with respect to plant height (Tables 5 and 6) and yield parameters (Tables 11 and 12), and recorded significantly lower final nematode populations (Tables 17 and 18).

The significantly better results recorded in plants of the LOCAL variety of soybean treated with powdered extract of *Hyptis suaveolens* may because the powdered botanical, *Hyptis suaveolens* used possibly interrupted the life cycle of *Meloidogyne incognita* halting the increase in their population and enhanced the growth of the plants. Izuogu *et al.* (2015) observed that there was 100% inhibition of egg-hatching and 100% juvenile mortality of root-knot nematode when tested with some botanicals. And in 2016, Izuogu *et al.* where in aqueous leaf extract of *Hyptis suaveolens* improved the vegetative growth of cowpea and possibly improved their photosynthetic ability. This is in agreement with the findings of Okechalu *et. al.* (2020), who reported that aqueous extract of *Hyptis suaveolens* improved the growth and yield of treated onions compared to their untreated controls.

Poor growth parameters and reduction in the yield of control plants may be attributed to the higher level of soil nematode population in these plants, probably, because they were not treated. Thus, this shows that the two varieties of soybean are susceptible or potential hosts to *Meloidogyne incognita,* and control measures should be directed at them.

The plants treated with *H. suaveolens* and *B. ferruginea* had lower final nematode population than the control counterpart (Tables 9 and 10). This is in agreement with the observation of Izuogu *et al.* (2016), who observed that *H. suaveolens* caused suppression and reduction of population density of the root-knot nematodes infecting cowpea due to their bioactive ingredients.

The effectiveness of *Hyptis suaveolens to* reduce nematode population as a result of reduced activities of nematode was due to nematicidal substances present in *Hyptis suaveolens*. The major compounds found from phytochemical screening of *Hyptis suaveolens* (Figure 1) were 3,7,11,15-tetramethyl-2-hexadecen-1-ol (12.05%), Phenol, 2,4-bis (1-phenylethyl) (6.14%), 1,2,4-oxidiazol-5(4H)-one, 4-(2-chorophenyl)-3-(3 pyridyl) (7.6%) and Squalene (22.25%) *(*Table 11). Whereas n-Hexadecanoic acid (6.99%), Hexadecanoic acid, Ethyl ester (33.54%), Phytol (27.10%), 9,12,15-Octadecatrienoic acid. 2,3-dihydroxylpropyl ester, (ZZZ) (21.45%) and Octadecanoic acid, ethyl ester (5.44%) were the major compounds found from phytochemical screening of *Bridelia ferruginea* (Figure 1; Table 12). In vitro studies by Denilson *et al.* (2019), proved that phenol compounds have both direct and indirect nematicidal activity on *Meloidogyne incognita.* Fujimoto *et al*. (2021) reported that exogenous application of Phytol inhibited the penetration of root-knot nematodes into arabidopsis plant although the Phytol did not exhibit any nematicidal activity. Finally, in 2020, Chun-Mei *et al*. reported that a novel strain of *Pseudomonas aureofaciens* produced nematicidal organic volatiles of which octanoic acid ethyl ester demonstrated the strongest nematocidal activity. These reports support the present study and implies that the nematicidal substances in *Hyptis suaveolens* including phenol and those of *Bradelia ferruginea*, Phytol and ethyl ester are responsible for the reduced nematode population density in treated plants.

Consequently, besides the powdery forms of *Hyptis suaveolens* that convincingly suppressed the nematodes, powdery forms of *Bradelia ferruginea* also gave impressive control of the nematodes. The combination of several plants with related compounds whose synergy is effective against nematodes may have an exciting nematicidal effect on nematodes. The high weight of pod and fruits in spite of RKN infection indicates the tolerance of the crop to nematodes aided by the presence of these treatments.

## CONCLUSIONS AND RECOMMENDATIONS

Soybean production, especially, in Nigeria, is constrained by *Meloidogyne incognita.* Powdered leaves of *Hyptis suaveolens* and *Bridelia ferruginea* are excellent nematocidal substances. Thus, the use of *Hyptis suaveolens* and *Bridelia ferruginea* as well as other plant materials could provide effective, eco-friendly and cheap method of controlling root-knot nematode infecting soybean. These plant materials have a wide range of mode of action that would suit possibly, every parasitic nematodes. The materials are readily available as unwanted plants, but could also be deliberately planted on non-arable lands to avoid competition with food crops for space.

Due to the observed increase in yield, reduction in nematode population density and the abundance of *H. suaveolens* as a weed, research should be focused on commercialization of *H. suaveolens*-based nematicides. The challenges impeding their formulation and commercialization should be urgently addressed to ease their adoption and utilization for sustainable plant-parasitic nematodes management. Additionally, the active ingredients in this weed plant could be synthesised to fasten the process and the formulation be made to suit every agro-ecological zone. Further, farmers should be enlightened to combine the powdery forms of *H. suaveolens* with other plant pesticides with similar synergistic effect as an integrated pest management strategy.

**Data Availability Statement:**

The data recorded on the growth and yield parameters, nematode population, and phytochemical screening are available if need be.

## REFERENCES

Ahnen, R.T., Jonnalagadda, S.S. and Slavin, J.L. (2019). Role of plant protein in nutrition, wellness, and health. Nutr. Rev., 77 (11) pp. 735-747

Anifowose, A.A., Izuogu, N.B. and Sossou, B.K., (2023). Susceptibility of sweet potato varieties to *Meloidogyne incognita* and use of effective microorganisms and compost manure for the disease management. *Pakistan Journal of Nematology*, 41(2): 135-143. DOI | https://dx.doi.org/10.17582/journal.pjn/2023/41.2.135.143

Bridge J, Page SLJ (1980): Estimation of root-knot nematode infestation levels on root using a rating chart. Tropical Pest Management, 26, 296–298.

Chun-Mei Z., Ming-Jie X., Yuan G , Xue-Wei L., Jin-Jin H , Sheng-Fang Z., Ke X., and Sheng Q. (2020). Identification and characterization of nematicidal activity of organic volatiles from a Pseudomonad rhizobacterium. Rhizosphere; Volume 16, December 2020, 100244. https://doi.org/10.1016/j.rhisph.2020.100244

Denilson F. Oliveira a, Viviane A. Costa a, Willian C. Terra b, Vicente P. Campos b, Pacelli M. Paula a, Samuel J. Martins c. (2019). mpact of phenolic compounds on Meloidogyne incognita in vitro and in tomato plants. Experimental Parasitology, Volume 199, April 2019, Pages 17-23. https://doi.org/10.1016/j.exppara.2019.02.009

FAOSTAT. 2019. "Soybean production in 2019, Crops/World regions/Production quantity". United Nations, Food and Agriculture Organization, Statistics Division, Retrieved February 8, 2021.

Fujimoto, T., Abe, H., Mizukubo, T., and Seo. S. (2021). Phytol, a Constituent of Chlorophyll, Induces Root-Knot Nematode Resistance in Arabidopsis via the Ethylene Signaling Pathway. Molecular Plant Microbe Interaction, Vol. 34, No. 3. https://doi.org/10.1094/MPMI-07-20-0186-R

Gema Nieto a, José M. and Lorenzo b c. (2022). Plant source: Vegetable oils. Sources, Health Implications, and Future Trends. Food Lipids, Pages 69-85. https://doi.org/10.1016/B978-0-12-823371-9.00011-3

Hussey, R.S. & Baker, K.R. (1973). 'Comparison of methods for collecting inocula of Meloidogyne spp., including a new technique'. Plant Disease Reporter, 57: 1025-1028.

Ichim, E., Marutescu, L., Popa, M., and Cristea, S. (2017).Antimicrobial efficacy of some plant extracts on bacterial ring rot pathogen, *Clavibacter michiganensis* ssp. sepedonicus. EuroBiotech. J., 1 pp. 93-96

Izuogu, N. B., Abolusoro, S. A., and Yakub, L. B. (2016) Nematicidal potential of aqueous extract of *Hyptis suaveolens* in the management of root-knot nematode, Meloidogyne incognita of some cowpea cultivars. Croat. Journal. Food Sci. Technol. (2016) 8 (1) 15-19

Izuogu, B., Yakubu, L.B. and Hinmikaiye, A.S. (2018). The effectiveness of dry leaf powder of *Phyllanthus amarus* Scumach and Thonn. and *Alstonia boone*i De Wild, on the root knot nematode, *Meloidogyne incognita* attacking tomato *Solanum lycopersicum* L. Var. Roma VF. Tropical Agriculture, 95(3). https://journals.sta.uwi.edu/ojs/index.php/ta/article/view/5384

Kankam, F. (2015). 'Effect of poultry manure on the growth, yield and root-knot nematode (*Meloidogyne* spp.) infestation of carrot (*Daucus carota* L.)'. *Archives of Phytopathology and Plant Protection.* Volume 48, Issue 5.

Nikkhah, M., Hashemi, M., Mohammad, B., Habibi, N., and Farhoosh, R. (2017), Synergistic effects of some essential oils against fungal spoilage on pear fruit Int. J. Food Microbiol., 257, pp. 285-294Neeraj, G.S., Kumar, A., Ram, S. and Kumar, V. (2017). Evaluation of nematicidal activity of ethanolic extracts of medicinal plants to *Meloidogyne incognita* (kofoid and white) chitwood under lab conditions. Int. J. Pure Appl. Biosci., 1 pp. 827-831

Mokrini, F., Janati1, S., Houari, A., Essarioui, A., Bouharroud1, R., Mimouni, A. (2018). Management of plant parasitic nematodes by means of organic amendment. Rev. Mar. Sci. Agron. Vét. (2018) 6 (3):337-344

NIST Chemistry Web Book, Mallard, W.G., Linstrom, P.J., Eds. NIST Standard Reference Database, National Institute of Standards and Technology, (https://webbook.nist.gov). (2008)

Nkechi, E.F., Ejike, O.G., Ihuoma, N.J. Maria-goretti, O.C., Francis, Godwin, U. N., and Njokuocha, R. (2018). Effects of aqueous and oil leaf extracts of Pterocarpus santalinoides on the maize weevil, Sitophilus zeamais, pest of stored maize grains. Afr. J. Agric. Res., 13, pp. 617-626

Okechalu, O.B., Zwalnan, N.D., Agaba, O.A. and Danahap, L.S. (2020). The efficacy of aqueous leaf extracts of *Hyptis suaveolens* (L.) Poit and *Ocimum basilicum* (L.) in the control of *Meloidogyne spp* infecting Onion, *Allium cepa* (L.). Bio-Research Vol. 18 No.1; pp. 1111-1119 (2020). ISSN (print): 1596-7409; eISSN (online):9876-5432

Oscar, S.A., Antonio, C.N., Marina, G.V., Elsa, R.S., and Gabriel, V.A. (2020). Phytochemical screening, antioxidant activity and in vitro biological evaluation of leaf extract of *Hyptis suaveolens* (L.) from South Mexico. S Afr J Bot. 2020;128:62–66. doi: 10.1016/Jsajb.2019.10.016. Stevenson, P.C. Isman, M.B. and Belmain, S.R. (2017). Pesticidal plants in Africa: a global vision of new biological control products from local uses. Ind. Crops Prod., 110 (2017), pp. 2-9

Prakash A, and Rao J. (2018). Botanical pesticides in agriculture. 3rd ed. CRC Press; Boca Raton, FL, USA: pp. 306–307. [Google Scholar]

Rizzo, G. and Baroni, L. (2018). Soy, soy foods and their role in vegetarian diets
Nutrients, 10 (1), p. 43

Sarker, S. and Nahar, L. (2012). Natural Products Isolation. Humana press, New York.

Sossou B.K., Izuogu, N.B., Anifowose, A.O., Ahamefule, H.E. (2022). 'Controlling root-knot nematode *Meloidogyne incognita* in tomatoes using modified effective microorganisms-fermented plant extract and compost manure'. *International Journal of Recycling of Organic Waste in Agriculture*; 11: 427-436 Doi:10.30486/IJROWA.2021.1937252.1307

Stevenson, P.C., Isman, M.B. and Belmain, S.R. (2017). Pesticidal plants in Africa: a global vision of new biological control products from local uses. Ind. Crops Prod., 110 , pp. 2-9

**Table 1: The effect of variety on the mean plant height of two soybean varieties on a nematode infested field**

| Varieties | 1WAP | 2WAP | 3WAP | 4WAP | 5WAP | 6WAP | 7WAP | 8WAP |
|---|---|---|---|---|---|---|---|---|
| TGXP1448 | 5.07a | 7.03b | 9.84b | 14.05a | 24.76a | 35.01a | 42.63a | 54.30a |
| LOCAL | 5.16a | 7.98a | 11.13a | 15.39a | 26.21a | 34.81a | 43.66a | 55.19a |
| SEM | 0.18 | 0.24 | 0.31 | 0.59 | 0.67 | 0.74 | 0.48 | 0.48 |

*Means with the same letter(s) are not significantly different according to fishers least significant difference at 5% level of significance. KEY: WAP= Week After Planting; Bridelia= Bridelia ferruginea; Hyptis = Hyptis suaveolens; SEM= Standard Error of Means, V1= TGXP1448; V2= LOCAL; M.I= Meloidogyne incognita; Pract= pratylenchus; Pop = Population.*

**Table 2: The effect of variety on the mean plant height of nematode-infected soybean varieties in the screenhouse**

| Varieties | 1WAP | 2WAP | 3WAP | 4WAP | 5WAP | 6WAP | 7WAP |
|---|---|---|---|---|---|---|---|
| TGXP1448 | 12.87a | 19.24b | 30.10b | 47.55b | 71.69b | 90.67b | 101.83a |
| LOCAL | 13.46a | 21.83a | 34.58a | 59.76a | 89.17a | 107.58a | 118.38a |
| SEM | 0.27 | 0.49 | 1.09 | 2.71 | 4.59 | 4.50 | 5.61 |

**Table 3: The effect of treatment on the mean plant height (cm) of *Glycine max* naturally infested by *Meloidogyne incognita* on the field**

| Treatment | 1WAP | 2WAP | 3WAP | 4WAP | 5WAP | 6WAP | 7WAP | 8WAP |
|---|---|---|---|---|---|---|---|---|
| *Bridelia* | 5.06a | 7.88a | 10.73b | 15.83a | 26.36a | 35.53a | 44.13a | 56.30a |
| *Hyptis* | 5.40a | 7.94a | 11.86a | 16.75a | 27.85a | 37.14a | 45.76a | 57.28a |
| Control | 4.88a | 6.71b | 8.88c | 11.59b | 22.24b | 32.06b | 39.55b | 50.66b |
| SEM | 0.22 | 0.29 | 0.38 | 0.723 | 0.818 | 0.91 | 0.59 | 0.59 |

**Table 4: The effect of treatment on the mean plant height of *Glycine max* infected with *Meloidogyne incognita* in the screenhouse.**

| Treatments | 1WAP | 2WAP | 3WAP | 4WAP | 5WAP | 6WAP | 7WAP |
|---|---|---|---|---|---|---|---|
| *Bridelia* | 12.69b | 20.99a | 34.63a | 58.78a | 89.28a | 110.50a | 114.71a |
| *Hyptis* | 14.71a | 22.41a | 35.00a | 59.69a | 88.75a | 107.13a | 122.94a |
| Control | 12.09b | 18.20b | 27.00b | 42.50b | 63.25b | 79.75b | 92.65b |
| SEM | 0.33 | 0.61 | 1.34 | 3.31 | 5.62 | 5.52 | 6.87 |

**Table 5: The effect of interaction between the treatment and variety on the mean plant height of two soybean varieties on a nematode infested field**

| Treatment | Varieties | 1WAP | 2WAP | 3WAP | 4WAP | 5WAP | 6WAP | 7WAP | 8WAP |
|---|---|---|---|---|---|---|---|---|---|
| *Bridelia* | V1 | 5.125a | 7.40abc | 9.93cd | 14.93ab | 25.20bc | 36.03ab | 43.75b | 55.70a |
| *Hyptis* | V2 | 5.00a | 8.35ab | 11.53ab | 16.73a | 27.53ab | 35.03abc | 44.50ab | 56.90a |
| *Bridelia* | V1 | 5.18a | 7.30bc | 10.75bc | 16.10a | 26.55ab | 36.48a | 45.23ab | 57.08a |
| *Hyptis* | V2 | 5.63a | 8.58a | 12.98a | 17.40a | 29.15a | 37.80a | 46.30a | 57.48a |
| Control | V1 | 4.90a | 6.40c | 8.85d | 11.13c | 22.52c | 32.53bc | 38.93c | 50.13b |
| | V2 | 4.85a | 7.03c | 8.90d | 12.05bc | 21.95c | 31.60c | 40.18c | 51.20b |
| SEM | | 0.31 | 0.41 | 0.53 | 1.02 | 1.16 | 1.29 | 0.84 | 0.84 |

**Table 6: The effect of interaction between the treatment and variety on the mean plant height of nematode-infected soybean varieties in the screenhouse**

| Treatment | Varieties | 1WAP | 2WAP | 3WAP | 4WAP | 5WAP | 6WAP | 7WAP |
|---|---|---|---|---|---|---|---|---|
| *Bridelia* | V1 | 12.50c | 19.88bc | 32.00ab | 49.28bc | 76.08bcd | 94.25bc | 101.40bc |
| | V2 | 12.88bc | 22.10ab | 37.25a | 68.28a | 102.50a | 119.50a | 128.03ab |
| *Hyptis* | V1 | 14.10ab | 21.00bc | 33.55ab | 55.88ab | 82.75abc | 101.50ab | 113.63abc |
| | V2 | 15.33a | 23.83a | 37.25a | 63.50a | 94.75ab | 120.00a | 132.25a |
| Control | V1 | 12.00c | 16.85d | 24.75c | 37.50c | 56.25d | 76.25c | 90.45c |
| | V2 | 12.18c | 19.55c | 29.25bc | 47.50bc | 70.25cd | 83.25bc | 94.85c |
| SEM | | 0.47 | 0.86 | 1.89 | 4.69 | 7.95 | 7.80 | 9.72 |

**Table 7: The effect of variety on the pod, fruit, root and shoot weight of soybean on nematode infested field**

| Varieties | Pod | Fruit | Root | Shoot |
|---|---|---|---|---|
| TGXP1448 | 2283.33a | 1825b | 182.08b | 343.75a |
| LOCAL | 2433.75a | 2100a | 132.08a | 363.75a |
| SEM | 73.9 | 70.6 | 12.5 | 14.6 |

**Table 8: The effect of varieties on the pod, fruit, root and shoot weight of nematode-infected soybean varieties in the screenhouse**

| Varieties | Pod weight | Fruit weight | Root | Shoot |
|---|---|---|---|---|
| TGXP1448 | 2.83b | 2.08b | 3.02b | 5.47b |
| Local | 3.33a | 2.70a | 2.28a | 6.53a |
| SEM | 0.08 | 0.11 | 0.10 | 0.105 |

*Means with the same letter(s) are not significantly different according to fishers least significant difference at 5% level of significance. KEY: WAP= Week After Planting; Bridelia= Bridelia ferruginea; Hyptis = Hyptis suaveolens; SEM= Standard Error of Means, V1= TGXP1448; V2= LOCAL; M.I= Meloidogyne incognita; Pract= pratylenchus; Pop = Population.*

**Table 9: The effect of treatment on mean weight of pod (grams) and mean weight of fruit (grams) per plant at harvest on the field**

| Treatments | Pod | Fruit | Root | Shoot |
|---|---|---|---|---|
| *Bridelia* | 2713.00a | 2112.5b | 156.875a | 461.88a |
| *Hyptis* | 2750.13a | 2412.5a | 146.250a | 374.38b |
| Control | 1612.50b | 1362.5c | 168.125b | 225.00c |
| SEM | 90.5 | 86.5 | 8.84 | 17.9 |

**Table 10: The effect of treatment on the mean pod, fruit, root and shoot weight of nematode-infected soybean at harvest in the screenhouse**

| Treatments | Pod | Fruit | Root | Shoot |
|---|---|---|---|---|
| *B. ferruginea* | 2.91b | 2.19b | 3.02b | 6.28b |
| *H. suaveolens* | 4.36a | 3.70a | 2.28a | 6.99a |
| Control | 1.98c | 1.75c | 3.99c | 4.74c |
| SEM | 0.10 | 0.14 | 0.12 | 0.12 |

**Table 11: The effect of interaction between the treatment and variety on the total yield for pod, fruit, root and shoot weight per plant at harvest on the field**

| Treatments | varieties | Pod | Fruit | Root | Shoot |
|---|---|---|---|---|---|
| *Bridelia* | TGXP1448 | 2475.00b | 1975b | 185.00b | 326.25b |
| *Bridelia* | LOCAL | 2775.00ab | 2250b | 128.75a | 422.50a |
| *Hyptis* | TGXP1448 | 2725.00ab | 2200b | 140.00a | 458.75a |
| *Hyptis* | LOCAL | 2951.25a | 2625a | 127.50a | 465.00a |
| Control | TGXP1448 | 1650.00c | 1300c | 196.25b | 210.00c |
| | LOCAL | 1575.00c | 1425c | 165.00b | 240.00c |
| SEM | | 15.8 | 12.2 | 7.22 | 25.3 |

**Table 12: The effect of interaction between the treatment and variety on the mean pod, fruit, root and shoot weight of nematode-infected soybean at harvest in the screenhouse**

| Treatments | Varieties | Pod | Fruit | Root | Shoot |
|---|---|---|---|---|---|
| *B. ferruginea* | TGXP1448 | 2.55c | 1.73d | 2.63c | 5.68c |
| | Local | 3.28b | 2.65c | 1.80ab | 6.88b |
| *H. suaveolens* | TGXP1448 | 4.20a | 3.35b | 2.13bc | 5.68c |
| | Local | 4.53a | 4.05a | 1.38a | 7.78a |
| Control | TGXP1448 | 1.75d | 1.40d | 4.30e | 4.95d |
| | Local | 2.20c | 1.18d | 3.68d | 4.53d |
| SEM | | 0.14 | 0.19 | 0.17 | 0.17 |

**Table 13: The effect of variety on nematode population for soil and root on the field**

| Soil nematode population | | | Root nematode population | | |
|---|---|---|---|---|---|
| **Varieties** | **Initial M.I pop.** | **Final M.I pop** | **Initial prat. pop** | **Final pract. pop** | **Final M.I pop** |
| V1 | 80.00 | 255.08b | 100.00 | 7.00b | 84.75b |
| V2 | 80.00 | 179.96a | 100.00 | 5.42a | 68.42a |
| SEM | 0.00 | 0.53 | 0.00 | 0.396 | 0.64 |

*Means with the same letter(s) are not significantly different according to fishers least significant difference at 5% level of significance. KEY: WAP= Week After Planting; Bridelia= Bridelia ferruginea; Hyptis = Hyptis suaveolens; SEM= Standard Error of Means, V1= TGXP1448; V2= LOCAL; M.I= Meloidogyne incognita; Pract= pratylenchus; Pop = Population.*

**Table 14: The effect of variety on nematode population for soil and root in the screenhouse**

| Soil nematode population | | | Root nematode population | |
|---|---|---|---|---|
| **Varieties** | **Initial M.I pop.** | **Mid M.I pop** | **Final M.I pop (root)** | **Final M.I pop (soil)** |
| V1 | 1000a0 | 551.83b | 594.92b | 253.54b |
| V2 | 1000a | 451.88a | 434.75a | 199.63a |
| SEM | 0.00 | 0.94 | 0.58 | 0.63 |

**Table 15: The effect of treatment on mean initial and final nematode population in soil and root on the field**

| Soil nematode population | | | | Root nematode population | | |
|---|---|---|---|---|---|---|
| **Treatments** | **Initial M.I pop.** | **Final M.I pop** | **Initial prat pop** | **Final pract pop** | **Final M.I pop** | **Final pract pop** |
| *Bridelia* | 80.00a | 20.44b | 100.00a | 3.00b | 14.75b | 3.00b |
| *Hyptis* | 80.00a | 14.75a | 100.00a | 0.13a | 10.00a | 0.13a |
| Control | 80.00a | 617.38c | 100.00a | 15.50c | 205.00c | 7.75c |
| SEM | 0.00 | 0.65 | 0.00 | 0.49 | 0.79 | 0.42 |

**Table 16: The effect of treatment on the initial and final nematode population in soil and root in the screenhouse**

| Soil nematode population | | Root nematode population | | |
|---|---|---|---|---|
| **Treatments** | **Initial M.I pop** | **Mid M.I pop** | **Final M.I pop** | **Final M.I pop** |
| *Bridelia* | 1000.00 | 155.19b | 75.0b | 49.56b |
| *Hyptis* | 1000.00 | 125.38a | 65.0a | 40.313a |
| Control | 1000.00 | 1225.00c | 1399.5c | 589.875c |
| SEM | 0.00 | 1.14 | 0.71 | 0.77 |

**Table 17: The effect of interaction between treatment and variety on nematode population for soil and root on the field**

| Treatments | varieties | Initial M.I pop. | Final M.I pop | Initial prat. pop | Final pract. pop | Final M.I pop | Final pract pop |
|---|---|---|---|---|---|---|---|
| *Bridelia* | V1 | 80.00a | 25.75d | 100.00a | 5.75b | 19.75d | 5.75b |
| | V2 | 80.00a | 15.13b | 100.00a | 0.25a | 9.75b | 0.25a |
| *Hyptis* | V1 | 80.00a | 19.75c | 100.00a | 0.00a | 14.75c | 0.00a |
| | V2 | 80.00a | 9.75a | 100.00a | 0.25a | 5.25a | 0.25a |
| Control | V1 | 80.00a | 719.75f | 100.00a | 15.25c | 219.75f | 9.75c |
| | V2 | 80.00a | 515.00e | 100.00a | 15.75c | 190.25e | 5.75b |
| SEM | | 0.00 | 0.93 | 0.00 | 0.69 | 1.11 | 0.59 |

**Table 18: The effect of interaction between treatment and variety on nematode population for soil and root in the screenhouse**

| Soil nematode population | | | | Root nematode population | |
|---|---|---|---|---|---|
| **Treatments** | **Varieties** | **Initial M.I pop** | **Mid M.I pop** | **Final M.I pop** | **Final M.I pop** |
| *Bridelia* | V1 | 1000.00a | 170.25d | 90.00d | 59.88d |
| | V2 | 1000.00a | 140.13c | 60.00b | 39.25b |
| *Hyptis* | V1 | 1000.00a | 134.75b | 75.25c | 50.75c |
| | V2 | 1000.00a | 116.00a | 44.75a | 29.88a |
| Control | V1 | 1000.00a | 1350.50f | 1599.50f | 650.00f |
| | V2 | 1000.00a | 1099.50e | 1199.50e | 529.75e |
| SEM | | 0.00 | 1.62 | 1.0 | 1.09 |

*Means with the same letter(s) are not significantly different according to fishers least significant difference at 5% level of significance. KEY: WAP= Week After Planting; Bridelia= Bridelia ferruginea; Hyptis = Hyptis suaveolens; SEM= Standard Error of Means, V1= TGXP1448; V2= LOCAL; M.I= Meloidogyne incognita; Pract= pratylenchus; Pop = Population.*

**Table 18: The effect of interaction between treatment and variety on nematode population for soil and root in the screenhouse**

| Soil nematode population | | | | Root nematode population | |
|---|---|---|---|---|---|
| Treatments | Varieties | Initial M.I pop | Mid M.I pop | Final M.I pop | Final M.I pop |
| *Bridelia* | V1 | 1000.00a | 170.25d | 90.00d | 59.88d |
| | V2 | 1000.00a | 140.13c | 60.00b | 39.25b |
| *Hyptis* | V1 | 1000.00a | 134.75b | 75.25c | 50.75c |
| | V2 | 1000.00a | 116.00a | 44.75a | 29.88a |
| Control | V1 | 1000.00a | 1350.50f | 1599.50f | 650.00f |
| | V2 | 1000.00a | 1099.50e | 1199.50e | 529.75e |
| SEM | | 0.00 | 1.62 | 1.0 | 1.09 |

**Table 19: Proposed Retention Time, Compounds and Peak Area (%) of *Hyptis suaveolens* using GC-MS**

| S. No. | Name of compounds | Retention time | Peak area % |
|---|---|---|---|
| 1 | Eucalyptol | 11.677 | 1.341 |
| 2 | Caryophyllene | 25.361 | 2.939 |
| 3 | Bergamotol, Z -alpha-trans | 33.507 | 0.961 |
| 4 | Bicyclo [3.1.1] heptane, 2,6,6 -trimethyl -, [1R-(1α,2β,5α)] | 37.294 | 3.897 |
| 5 | 3,7,11,15 -tetramethyl -2-hexadecen -1-ol | 37.820 | 1.336 |
| 6 | n-Hexadecanoic acid | 38.770 | 3.565 |
| 7 | Hexadecanoic acid, ethyl ester | 38.872 | 1.293 |
| 8 | Naphthalene, decahydro -1,1,4a-trimethyl -6-methylene -5-(3-methylene) - | 38.951 | 1.008 |
| 9 | 1,3,6,10 -Cyclotradecatetraene, 3,7,11 -timethyl -14-(1-methylethyl) - [S-(E,Z,E,E)] - | 39.438 | 0.894 |
| 10 | 3,7,11,15 -tetramethyl -2-hexadecen -1-ol | 39.682 | 12.052 |
| 11 | 9,12,15 -Octadecatrienoic acid, 2,3 -dihydropropyl ester, (Z,Z,Z) - | 39.917 | 4.436 |
| 12 | Octadecanoic acid, ethyl ester | 40.114 | 0.798 |
| 13 | Phytol, acetate | 40.225 | 1.807 |
| 14 | Cyclotetradecane | 40.483 | 1.233 |
| 15 | 1H-Benzotriazole, 1 -ethenyl - | 40.6955 | 0.748 |
| 16 | Benzene, 1 -(1-buten-3-yl) -4-pentyl | 40.758 | 5.688 |
| 17 | 10H- Phenoxaphosphine, 2,8 -diethyl-10-hydroxy-, 10-oxide | 40.821 | 12.169 |
| 18 | 1-Phenananthrenemethanol, 1,2,3,4,4a,9,10.10a -octahydro -1, 4a-dimethyl -7-(1-methylethyl) - | 41.041 | 2.889 |
| 19 | Abietic acid | 41.221 | 3.474 |
| 20 | Phenol, 2,4 -bis(1-phenylethyl) - | 41.308 | 6.136 |
| 21 | 1-Heptatriacotanol | 41.708 | 1.239 |
| 22 | 1,2,4-oxidiazol -5(4H)-one, 4-(2-chlorophenyl) -3-(3-pyridyl) - | 42.117 | 7.670 |
| 23 | Squalene | 44.717 | 22.427 |

*Means with the same letter(s) are not significantly different according to fishers least significant difference at 5% level of significance. KEY: WAP= Week After Planting; Bridelia= Bridelia ferruginea; Hyptis = Hyptis suaveolens; SEM= Standard Error of Means, V1= TGXP1448; V2= LOCAL; M.I= Meloidogyne incognita; Pract= pratylenchus; Pop = Population.*

**Table 20: Proposed Retention Time, Compounds and Peak Area (%) of *Bridelia ferruginea* using GC-MS**

| S. No. | Name of compound | Retention time | Peak area % |
|---|---|---|---|
| 1 | 2-Fluoro-4-(trifluoromethyl)acetophenone | 24.341 | 1.024 |
| 2 | 2-6-Pentadecen-1-ol acetate | 36.351 | 1.653 |
| 3 | n-Hexadecanoic acid | 37.696 | 6.987 |
| 4 | Hexadecanoic acid, ethyl ester | 37.802 | 33.544 |
| 5 | Phytol | 38.578 | 27.111 |
| 6 | Linoleic acid ethyl ester | 38.834 | 2.790 |
| 7 | 9,12,15-Octadecatrienoic acid, 2,3-dihydroxypropyl ester, (Z, Z, Z)- | 38.872 | 21.452 |
| 8 | Octadecanoic acid, ethyl ester | 39.009 | 5.439 |