



### Full Length Article

## Nematicidal Effects of *Acacia nilotica*, *Azadirachta indica*, *Brassica chinensis* and *Ecklonia maxima* against Soybean Cysts Nematode

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### Abstract

The effect produced by aqueous extract of *Acacia nilotica*, *Azadirachta indica*, *Brassica chinensis*, and *Ecklonia maxima* in the control of soybean cysts nematode (SCN) was tested in laboratory and green house. SCN eggs, second stage juvenile and soybean plants inoculated with 1000 eggs were treated with extract of each of the plant material at concentrations of 120 µg/mL and 12 µg/mL. Results obtained showed all plant materials significantly reduced *H. glycine* population compared to control. *A. indica* at concentration of 120 µg/mL exhibited highest inhibition to egg hatch, juvenile mortality, reduction in number of females and cysts of 75.21%, 100%, 83.12% and 80.17%, respectively. This was closely followed by *B. chinensis*, *A. nilotica* and *E. maxima*. The aim is to study the efficacy of each plant material for the possibility of mixing their crude form to formulate an organic nematicide alternative to methyl-bromide that was complete banned globally in 2005 due to its harmful effect on the ozone layer. *A. indica* and *B. chinensis* showed high potential nematicidal properties, which could be explored further in effort to formulate organic nematicide from mixture of two the two plant materials in order to benefit from synergistic and potentiating effect. © 2013 Friends Science Publishers

**Keywords:** *Heterodera glycines*; *Glycine max*; Alternative control

### Introduction

Currently, the cyst nematodes (*Heterodera* spp.) are considered most important soil borne pathogens of crops throughout the world (Wrather and Mitchum, 2010; Hajihassani *et al.*, 2011). Soybean cysts nematode (*Heterodera glycines* Ichinohe, 1952) is considered a devastating pest in soybean growing areas of the world (Wrather and Mitchum, 2010). Being a cyst producing nematode, it has a high survival and dissemination capacity, making its control difficult. Chemical nematicides are the main control method used by farmers. Nematicides although efficient and drastic in action are now being reappraised due to hazards they pose to the environment. De-registration of some of the more hazardous nematicides, for instance, the world wide phasing out of methyl bromide completed in 2005 due to its effect on the atmospheric ozone layer has removed the charm many growers relied upon for over forty years for control of plant parasitic nematodes (Sikora, 2002).

Discovery of plant materials based nematicidal substances lend a boost to safe and sustainable alternative approaches for nematodes control (Chitwood, 2002; Rehman *et al.*, 2006; Safdar *et al.*, 2012). Numerous biocidal “active principles” extracted from plants have

exhibited efficacy against different types of pathogens including plant parasitic nematodes (Bones and Rossiter, 2006; Kabeh and Jalingo, 2007; Elbadri *et al.*, 2009; Khan *et al.*, 2009; Sultana *et al.*, 2011). However, there is comparatively little information on use of plant materials to control cyst forming nematodes on field crops such as cereal and pulses. Therefore, the objective of this study is to assess the effect of applying cold aqueous extracts of acacia (*Acacia nilotica*), Chinese cabbage (*Brassica chinensis*), neem (*Azadirachta indica*), and sea bamboo (*Ecklonia maxima*) in suppressing population of soybean cyst nematode (*Heterodera glycines*) and to evaluate the concentration of materials needed to effectively cause reduction un population of *H. glycines* without any phyto-toxicity effects on the crop.

### Materials and Methods

Milled *A. indica* kernel, *A. nilotica* resin, *B. chinensis* leaves and *E. maxima* were bought from China Ocean University Organic Fertilizer Manufacturing Company, Qingdao, China. Each plant material was weighed, 30 grams, wrapped in a 1 mm diameter mesh cloth and immersed in 200 mls of sterilized distilled water (SDW) and allow to stay for 24

hours (Kumar, 2003). The water extract was obtained by filtering the mixture (plant material and SDW) through a Whatman # 42 filter paper (Iqbal *et al.*, 2001). Filtrates were made up to 250 mls using SDW to give a concentrations of 0.12 mg/L, which were subsequently referred to as standard concentrations (SC) and were serially diluted with SDW to give two concentrations of 120 µg/mL and 12 µg/mL used in experiments. Soybean variety highly susceptible to *H. glycines* ("Mudanjiang GB1352") was planted in *H. glycines* infested soil to culture the inoculums. Whenever the need arise, soybean which was uprooted and matured cyst collected from the plant roots and soil. The *H. glycines* cysts were extracted from the soil sample using a modified fenwick can (MFC) flotation method (Fenwick, 1940). To prepare the test plant, soybean seeds are placed on a large petri-dish covered with a lid and placed inside a growth chamber (25-27°C), germinated seeds were used in pot experiments.

Laboratory experiments were conducted to study the mechanism of nematodes suppression by the four plant aqueous extracts. Aliquots of 4 mls filtrates of each of the plant material at concentrations of 120 µg/mL and 12 µg/mL and 1 mL of SDW containing 200 eggs/mL of *H. glycines* suspension was loaded into Bureau of Plant Industry (BPI). Each BPI dish was placed inside a petri-dish (6 cm diameter) and covered with lid. SDW was used in place of extracts for control. To inhibit bacterial attack on the eggs, 0.3 mL of 1.0% streptomycin sulfate was added to each treatment (Hassan *et al.*, 1981). Petri-dishes containing treatments were arranged in complete randomized design (CRD) inside a growth chamber at temperature of 25-27°C. Hatched second stage juveniles (J2) of *H. glycines* were counted under a light microscope at intervals of 48, 60, 72, 84 and 96 h. The experiment was replicated four times and repeated twice. Percentage *H. glycines* egg, hatch inhibition was determined using formula shown below:

$$\text{Percentage egg inhibition (\%)} = \frac{1 - (N \text{ in T after treatment})}{N \text{ in Control after treatment}} \times 100$$

Where: N = Number of eggs; T = Treated.

A second experiment was conducted to test the effect of the extracts on *H. glycines* juveniles. Aliquots of 4 mls of each plant material at concentrations of 120 µg/mL and 12 µg/mL and 1 mls SDW of *H. glycines* juveniles suspension containing 20 J2s were loaded into a BPI dishes, these were incubated inside a growth chamber at 26 - 28°C. Treatments were observed under a light microscope and a picking hair was used to slightly touch the J2, those that did not respond to the touching sensation were counted as dead at time intervals of 48, 60, 72 and 84 h. Sterilized distilled water was used to replace extract as control. The experiment was replicated four times and repeated twice. Percentage J2 mortality was calculated using the formula shown below:

$$\text{Percentage J2 mortality (\%)} = \frac{(\text{Mortality \% in Treated} - \text{Mortality \% in Control})}{100 - \text{Mortality \% in Control}} \times 100$$

In green house soybean seedlings were transplanted at the rate of one seed per pot (20 cm in diameter, 15 cm deep) containing a mixture of sterilized sandy soil (50 % sand, 30% loam and 20% clay). Each plant was inoculated with 1000 eggs of *H. glycines*. A period of 1 week is allowed to elapse after inoculation and 100 mls of extracts were used to drench the soil in place of tap water supply. Distilled water only served as control. Eight weeks after planting, soybeans were up rooted, roots were excised from shoots and washed in a gently flow of tap water. Using a stereomicroscope the number of *H. glycines* females and cysts were counted per one gram root per plant. Data was taken shoot height, root length, dry shoot weight and dry root length. The experiment was replicated four times and repeated twice.

## Data Analysis

Data were analyzed using a one- way analysis of variance (ANOVA) conducted using Michigan State University statistical software (MSTAT-C, 2000). Significant differences among the treatment means was separated using Fisher's protected least significant difference (LSD) at 95% level of confidence.

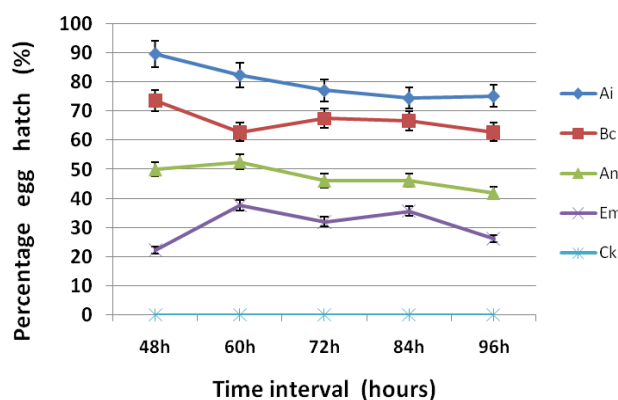
## Results

Aqueous extracts of all four plant materials at concentrations of 120 µg/mL hindered significantly ( $P < 0.05$ ) *H. glycines* egg hatch compared to control. Percentage egg hatch inhibition after interval of 96 hours for *A. indica*, *B. chinensis*, *A. nilotica* and *E. maxima* was 75.21%, 62.90%, 41.80% and 26.23%, respectively (Fig. 1a). Similar trend, although at lower percentage inhibition was observed at low concentration of 12 µg/mL. SDW control treatment did not hinder egg hatch.

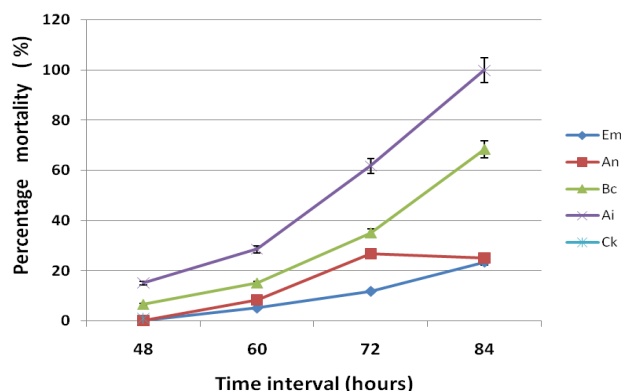
All plant material extracts significantly ( $P < 0.05$ ) differ from, and caused higher percentage mortality of J2 than the control. Aqueous extract of *A. indica* at concentration of 120 µg/mL at an interval of 84 h caused 100% percentage mortality of J2, this is followed by *B. chinensis*, *A. nilotica* and *E. maxima* that caused J2 mortality of 68.34%, 43.34% and 23.34%, respectively (Fig. 1b). Similar trend was observed at lower concentration of 12 µg/mL.

## Effect of Extracts on *H. glycines* and Soybean

Application of all four plant material aqueous extracts at concentrations of 120 µg/mL and 12 µg/mL significantly ( $P < 0.05$ ) hindered *H. glycines* development on soybean roots compared to control. At concentration of 120 µg/mL, percentage *H. glycines* female development hindrance of *A. indica*, *B. chinensis*, *A. nilotica* and *E. maxima* were 83.12, 66.23, 50.65 and 40.26%, respectively compared to control treatment that exhibit 0% hindrance to female development. Similar trend was observed at the low concentration of 12 µg/mL (Fig. 2).



**Fig. 1a:** Percentage inhibition of *Heterodera glycines* egg hatch after treatment with *Azadirachta indica* (Ai), *Brassica chinensis* (Bc), *Acacia nilotica* (An) and *Ecklonia maxima* (Em) aqueous extracts at concentration of 120  $\mu\text{g/mL}$ . Control= sterilized distilled water (SDW). Values are means of two trials with four replicates each



**Fig. 1b:** Percentage juvenile mortality of *Heterodera glycines* after treatment with *Azadirachta indica* (Ai), *Brassica chinensis* (Bc), *Acacia nilotica* (An) and *Ecklonia maxima* (Em) aqueous extracts at concentration of 120  $\mu\text{g/mL}$ . Control = sterilized distilled water (SDW). Values are means of two trials with four replicates each

Soybean growth parameters of shoot height, root length, dry shoot and root weights at treatment concentration of 120  $\mu\text{g/mL}$  of all four plant materials were significantly ( $P < 0.05$ ) increased compared to control treatment. However, *A. indica* exhibited significantly ( $P < 0.05$ ) highest mean shoot height of 68.7 cm, followed by *E. maxima*, *B. chinensis* and *A. nilotica*, which gave mean shoot heights of 61.2 cm, 59.7 cm and 43.2 cm compared to control treatment, which gave significantly ( $P < 0.05$ ) the lowest mean shoot height of 32.3 cm. Other growth parameters of mean root length, dry shoot and root weight more or less follow a similar trend as showed in Table 1.

## Discussion

This study has demonstrated all four plant aqueous extracts

**Table 1:** Effect of plant materials on growth of soybean inoculated with 1000 eggs of *H. glycines*

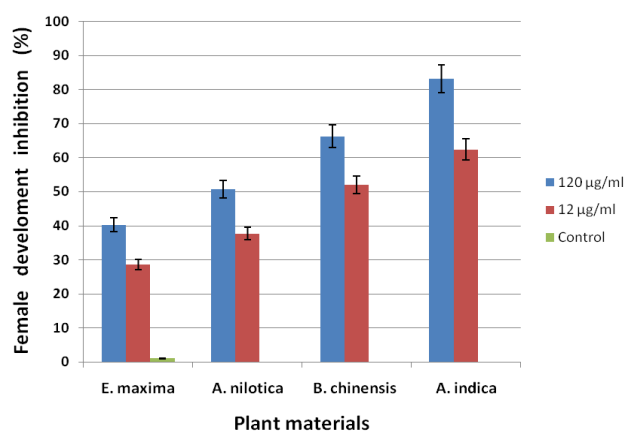
Plant material	Conc. ( $\mu\text{g/mL}$ )	Mean Shoot Height (cm)	Mean Root Length (cm)	Dry shoot Weight (g)	Dry root Weight (g)
An 120		43.22 <sup>d</sup>	10.50 <sup>bc</sup>	0.60 <sup>d</sup>	0.33 <sup>d</sup>
An 12		36.73 <sup>e</sup>	8.98 <sup>d</sup>	0.48 <sup>e</sup>	0.26 <sup>e</sup>
Bc 120		59.68 <sup>b</sup>	11.43 <sup>b</sup>	1.25 <sup>ab</sup>	0.45 <sup>c</sup>
Bc 12		54.33 <sup>c</sup>	9.63 <sup>c</sup>	0.85 <sup>c</sup>	0.35 <sup>d</sup>
Ai 120		68.70 <sup>a</sup>	13.80 <sup>a</sup>	1.40 <sup>a</sup>	0.65 <sup>a</sup>
Ai 12		58.18 <sup>b</sup>	9.95 <sup>c</sup>	0.93 <sup>c</sup>	0.47 <sup>c</sup>
Em 120		61.25 <sup>ab</sup>	11.30 <sup>b</sup>	1.08 <sup>b</sup>	0.53 <sup>b</sup>
Em 12		54.58 <sup>c</sup>	9.83 <sup>c</sup>	0.83 <sup>c</sup>	0.33 <sup>d</sup>
Control		32.30 <sup>e</sup>	7.87 <sup>e</sup>	0.40 <sup>e</sup>	0.18 <sup>e</sup>

Each value is a mean of two trials with four replicates

Means followed by the same letter within each column are not significantly different ( $P < 0.05$ ) as indicated by Fisher's least significant difference (LSD) test

An= *Acacia nilotica*; Bc = *Brassica chinensis*; Ai= *Azadirachta indica*; Em= *Ecklonia maxima*

Control = Sterilized distilled water (SDW)



**Fig. 2:** Percentage female development inhibition after treatment with *Azadirachta indica* (Ai), *Brassica chinensis* (Bc), *Acacia nilotica* (An) and *Ecklonia maxima* (Em) aqueous extracts at concentration of 12  $\mu\text{g/mL}$  and 120  $\mu\text{g/mL}$ . Control= Sterilized distilled water (SDW). Each value is a mean of two trials with four replicates

of plant materials significantly ( $P < 0.05$ ) more effective in inhibiting egg hatch, causing J2 mortality and reducing number of cysts and females of *H. glycines* compared to control. Nevertheless, *A. indica* and *B. chinensis* exhibited significantly ( $P < 0.05$ ) more nematocidal properties against *H. glycines* population compared to *A. nilotica* and *E. maxima*. The pot experiments showed all four plant material extracts to a different extent reduced *H. glycines* cysts and female numbers; this apparently caused an increase in growth parameters of the soybean. The study revealed that type of plant from which the extract was produced influenced to large extends the activity of the plant aqueous extracts against the *H. glycines* population, a similar observation made on *M. incognita* J2 (Rotim and Moens, 2005). The study also indicated that different concentration of the plant aqueous extracts exhibited different nematocidal efficacy.

This nematocidal activity is related to presences of several triterpenoids present in the plant seeds such as azadirachtin A, Azadirachtin B, Azadirachtin H, desacetylnimbin, nimbin, salannin. Plants in the genus *Brassica* spp. all contain glucosinolates (Fahey *et al.*, 2001), which are hydrolyzed by specific endogenous thioglucosidases to yield a variety of biologically active products, including nitriles, thiocyanates, and isothiocyanates – ITCs (Bones and Rossiter, 2006). ITCs are related to compounds found in chemical soil fumigants metham sodium and dazomet, which release methyl isothiocyanate into the soil; this could be a valuable component of a methyl bromide alternatives program (Fahey *et al.*, 2001). In the laboratory experiments, extracts of *A. nilotica* at concentration of 120 µg/mL showed promising nematocidal effect and ranked third after *A. indica* and *B. chinensis*. This is in consonance with a study conducted by Sultana *et al.* (2011) on the control of *Meloidogyne* spp. using *A. nilotica* whereby 100% J2 mortality was obtained after 48 h exposure. In the pot experiments *A. nilotica* reduced the number of *H. glycines* cysts and females by half at high concentration of 120 µg/mL; this is in conformity with a green house pot study conducted by Elbadri *et al.* (2009) on control of *M. incognita* on water melon *Citrullus lanatus* Thunb.). Sea bamboo is the least in nematocidal properties. Paradoxically, it caused high increase in growth parameters of soybean second only to neem. This contradictory situation can be attributed to the fact that sea bamboo add nutrients to the soybean compared to extracts of the other plant materials. This makes the soybean more robust and tolerant to the *H. glycines* attack. This fact as elucidated by Oka (2010) showed addition of organic amendments somehow makes plants more tolerant or resistance to parasitism by nematodes. Sea bamboo has been implied to induce resistance to abiotic and biotic stresses including nematodes parasitism (Khan *et al.*, 2009).

In conclusion, *A. indica* and *B. chinensis* have immense potential to contribute to the integrated management of *H. glycines* population on soybean. Further research is essential in order to quantify the efficacy of the plant materials under field conditions to enhance its utilization. This can be explored further in effort to formulate organic nematocide from a mixture of two or more plant material in order to benefit from synergistic and potentiating effect of a mixture of different materials.

## Acknowledgements

The research was supported by Special Fund for Agro-scientific Research in the Public Interest in China (No, 200903040 and 201103018), and the authors wish to express their profound gratitude to Chinese government (CSC No. 2008566013) for conduct this study.

## References

- Bones, A.M. and J.T. Rossiter, 2006. The enzymatically and chemically induced decomposition of glucosinolates. *Phytochemistry*, 67: 1053–1067
- Chitwood, D.G., 2002. Phytochemical based strategies for nematodes control. *Annu. Rev. Phytopathol.*, 40: 221–249
- Elbadri, G.A.A., D.W. Lee, J.C. Park and Y.H. Choo, 2009. Nematicidal efficacy of herbal powders on *Meloidogyne incognita* (Tylenchida: Meloidogynidae) on potted watermelon. *J. Asia-Pacific Entomol.*, 12: 37–39
- Fahey, J.W., A.T., Zalcmann and P. Talalay, 2001. The chemical diversity and distribution of glucosinolates and isothiocyanates among plants. *Phytochemistry*, 56: 5–51
- Fenwick, D.W., 1940. Methods for the recovery and counting of cysts of *Heterodera schachtii* from soil. *J. Helminth.*, 18: 155–172
- Hajihassani, A., Z.T. Maafi, A. Ahmadi and M.Taji, 2011. Survey and biology of cereal cyst nematodes, *Heterodera latipons*, in rain fed wheat in Markazi Province, Iran. *Int. J. Agric. Biol.*, 13: 576–580
- Hassan, Y.M., S.I. Hussain and A.J. Al-Zarari, 1981. Effect of some plant extracts of some poisonous plants of Iraq on mortality of citrus nematodes. *Acta Bot. Ind.*, 9: 198–200
- Iqbal, Z., M.A. Munir, M.N. Khan, M.S. Akhtar and I. Javed, 2001. *In vitro* inhibitory effects of sorghum bicolor on hatching and moulting of *Haemonchus contortus* eggs. *Int. J. Agric. Biol.*, 3: 451–453
- Kabeh, J.D. and M.G.D.S.S. Jalingo, 2007. Exploiting neem (*Azadirachta indica*) resources for improving the quality of life in Taraba State, Nigeria. *Int. J. Agric. Biol.*, 9: 530–532
- Khan, W., U.P. Rayirath, S. Sumanian, N. Mundaya, M.N. Jithesh, P. Rayorath, D.M. Hodges, A.T. Crithley, J.S. Craigie, J. Norrie and B. Prithiviraj, 2009. Sea bamboo extracts as bio-stimulants of plant growth and development. *J. Plant Growth Regul.*, 28: 386–399
- Kumar, J., 2003. *La Lute Contre Les Insectes Ravageurs Et Les Champignons Pathogènes, La Situation De L'agriculture Africaine*, pp: 314–344. Pars, CTA Kathala
- Oka, Y., 2010. Mechanism of nematodes suppression by organic soil amendments- A review. *App. Soil Ecol.*, 44: 101–115
- Rehman, A., R. Bibi and M.H. Ullah, 2006. Evaluation of different chemicals against root knot nematode (*Meloidogyne incognita*) on sunflower. *J. Agric. Soc. Sci.*, 2: 185–186
- Rotim, O. and M. Moens, 2005. Effects of leaf extracts of some herbs on the juveniles of *Meloidogyne incognita* (Kofoid and White) Chidwood. *Nig. J. Plant. Prot.*, 22: 95–104
- Safdar, H., N. Javed, S.A. Khan, I. Haq, A. Safdar and N.A. Khan, 2012. Control of *Meloidogyne incognita* (Kofoid and White) chitwood by cadusafos (Rugby®) on tomato. *Pak. J. Zool.*, 44: 1703–1710
- Sikora, R.A., 2002. Strategies for biological system management of nematodes in horticultural crops – Fumigate, confuse or ignore them. *Appl. Biol. Sci.*, 67: 3–15
- Sultana, N., M. Akhter, M. Saleem and A.Y. Yousaf and A.Y. 2011. Nematicidal effect of *Acacia nilotica* and *Gymnema sylvestris* against second stage juveniles of *Meloidogyne incognita*. *J. Entomol. Nematol.*, 3: 25–29
- Wrather, A. and M. Mitchum, 2010. *Soybean Cyst Nematode: Diagnosis and Management*. University of Missouri Extension bull. Page 1. <http://extension.missouri.edu/p/G4450> (Last visited in March, 2012)

(Received 17 September 2012; Accepted 04 December 2012)