Bajopas Volume 4 Number 2 December, 2011 http://dx.doi.org/10.4314/bajopas.v4i2.34

Bayero Journal of Pure and Applied Sciences, 4(2): 169 – 172
Received: September, 2011
Accepted: December, 2011
ISSN 2006 – 6996

INHIBITION OF GERMINATION AND GROWTH BEHAVIOR OF SOME COWPEA VARIETIES USING NEEM (*AZADIRACTA INDICA)* LEAF WATER EXTRACTS

***Lawan, S. A.[1]., Suleiman, M.[1] and S. U. Yahaya[2]**
[1]Biological Science Department, College of Arts, Science and Remedial Studies, Kano.
[2]Crop Science Department, Kano University of Science and Technology, Wudil Kano.
*Correspondence author: lawansaniahmed@yahoo.com

ABSTRACT
The experiment was conducted to observe the inhibitory effects of the leaf extract derived from neem (Azadiracta indica) on germination and growth behavior of some cowpea varieties (receptor). Experiments were set on sterilized petridishes with a photoperiod of 24 hours at room temperature of 27-30^{o}C. The effects of the different concentrations of aqueous extracts were compared with distilled water (control). The aqueous extracts of leaf caused significant inhibitory effects on germination, root and shoot elongation and development of lateral roots of treated plants. Bioassays indicated that the inhibitory effect was proportional to the concentrations of the extract, as concentration increased the extent of inhibition also increased. The study also revealed that inhibitory effect was much pronounced in root and lateral root development rather than germination and shoot growth.

Keywords: Neem, Allelopathic effect, Leaf extract, Germination, Growth behavior

INTRODUCTION

Allelopathy has been defined as an adverse influence of one plant or microorganism on another (Rice, 1984). In agricultural practice, allelopathy is exploited for weed control (Kohli *et al.,* 1998). Association and dissociation pattern between certain plant species are widely known. Such phenomenon may be governed by direct competition for necessary growth factors or through addition of allelopathic chemicals into the soil environment (Einhelling, 1996; Ashrafi *et al.,* 2007). It has been documented that allelopathy may play an important role in plant-plant interference by those chemical compounds (Inderjit and Dakshini, 1992). Some of those compounds are released into the environment through leaching, litter decomposition, root exudation or direct volatilization, and could affect (either positively or negatively) germination and growth of other species (Gross and Parthier, 1994; Seligler, 1996). A number of weed and crop species have been reported to possess allelopathic effects on the growth of other plant species (Rice, 1984). Chemicals with inhibitory activity are present in many plants and in many organs, including leaves, flowers, fruits and buds (Inderjit, 1996; Ashrafi *et al.,* 2007).

The need to reduce harmful environmental effects from the overuse of herbicides has encouraged the development of weed management systems which are dependent on ecological manipulations rather than agrochemicals (Liebman and Ohno, 1997; Zoheir *et al.,* 2008; Zoheir *et al.,* 2009). *Azadiracta indica,* or Neem Tree, is an evergreen tree native to Southeast Asia. All parts of the tree have been used medicinally for centuries. It is widely used in toothpastes, soaps and lotion today, as well as biological insecticide. *Azadiracta* is a genus of two species of trees in the flowering plant family Meliaceae. Numerous species have been described in the genus but only two are currently recognized, *A. excels* (Jack) Jacobs, and the economically important Neem tree, *A. indica* A. Juss. (Mabberley, 1995; Ashrafi *et al*., 2008). Neem (*Azadiracta indica,* A. Juss) is a versatile tree native to South and Southeast Asia, Japan, tropical USA, South America, Australia and Africa (Bokhari and Aslam, 1985; Von Maydell, 1986). The tree was introduced into Nigeria from Ghana, and it was first grown from the seed in Maiduguri, the then Bornu Province (now Borno State), Nigeria, in 1928 (National Research Council, 1992; Nwoekeabia, 1994). Neem is a moderate sized to large, usually evergreen tree, with a fairly dense crown and glabrous leaves divided into leaflets. The bark is fairly thick, furrowed longitudinally or obliquely and is dark grey outside and reddish brown inside. The tree produces flowers throughout the year but fruits during the cold harmattan season which corresponds with the winter of temperate climates. The fruits are yellowish green when ripe and have a sweetish pulp containing one seed. However, these properties have been attributed to hundreds of chemicals present in the tree (Sankaram, 1987). In Northern Nigeria, its various plant parts have been traditionally used to control domestic insects- pests in stored grains, crop pests, livestock medicines and the treatment of general body pain after child delivery, pyorrhea, and intestinal worms (Bokhari and Aslam, 1985). Because neem may contain a number of useful chemicals with multiple uses and adaptability to diverse habitat and climatic conditions, interest in the tree has increased. However, very few reports or literatures available concerning the allelopathic potential of tropical and subtropical plants like Neem (*Azadiracta indica*) have been published.

This study was conducted to determine the inhibitory influence of this plant species on some cowpea varieties using different concentrations of leaf extracts obtained under laboratory conditions.

MATERIALS AND METHODS

Mature leaves of *Azadiracta indica* were collected and identified from College of Arts, Science and Remedial Studies, campus, Kano. Located on (Lat. 12^0 03'N; Long. 8^0 32'E) in the Sudan Savanna Agro-ecological region of Nigeria (Olofin, 1987). This sample was air dried at room temperature for two weeks. The dried leaves were ground into fine powder in a mortar and pestle. The seeds of cowpea varieties used in this research were Dan'ila, Yaro da kokari, Kanannado, Aloka and Dan-eka obtained from International Institute of Tropical Agriculture (IITA) Kano.

Preparation of Aqueous Extracts

The dried leaves were grounded to fine powder using motar and pestle. Ten grammes (10 g) of the powder was weighed and soaked in 1000 cm^3 of distilled water for 24 hours. The solution was filtered through double layer of muslin cloth followed by No.1 Whatman filter paper. Five concentration of various extracts were made, (10, 20, 30, 40 and 50 %) from the leaf extract and stored separetly in cornical flasks. Distilled water was used as control (0 %) as described by Jafari *et al.* (2007).

Treatments

The following treatments were used in the experiment.

T_0 = Seeds of receptor plants grown in distilled water only (control)

T_1 = Seeds of receptor plants grown in leaf extracts of 10 % concentrations

T_2 = Seeds of receptor plants grown in leaf extracts of 20 % concentrations

T_3 = Seeds of receptor plants grown in leaf extracts of 30 % concentrations

T_4 = Seeds of receptor plants grown in leaf extracts of 40 % concentrations

T_5 = Seeds of receptor plants grown in leaf extracts of 50 % concentrations

Germination and Growth Records

The experiment covered a period of ten days to allow the last seed germination and the measurement of the shoot and root length. The germination test was carried out in sterile petridishes of 12 cm in diameter placing a Whatman No.3 filter paper on the petridishes. The extract of each concentration was added to each petridish of the respective treatment daily in order to keep the seeds moist. The control was treated with distilled water only. Ten seeds of each cowpea variety were placed in the petridish replicated five times and arranged in complete randomized design. The germination was recorded daily and the seeds were considered as germinated when the radicle emerged.

Germination Count and Growth Parameters

The results of the experiment were determined by counting the number of germinated seeds, number of lateral roots and measuring the length of primary root and main shoot on 10^{th} day of the experiment. The data were subjected to analysis of variance (ANOVA). Significant means were separated using Duncan's Multiple Range Test (DMRT) (Mukhtar, 2003).

Calculation of inhibitory effects

The percentage of inhibitory effects of the extracts on germination and growth parameters of treatment plants to control was calculated using formula described by Sundra and Pote (1978).

$$I = 100 - (E_2 \times 100/E_1)$$

Where,

I = % inhibition.

E_1 = Response of control plant.

E_2 = Response of treatment plant.

RESULTS AND DISCUSSION

The germination percentage of the five cowpea variety plants is shown in Table 1. In most cases, the variation in the result of germination percentage is due to concentration differences. With the increase of concentration, the inhibitory effect was progressively increased. Highest inhibitory effect was exerted by T_5 treatment in all cases except in Dan'ila variety. Among the survivors, the highest inhibitory effect (-98.3%) was recorded from Dan-eka, this is followed by Yaro da kokari with (-98.0%) all at T_5 treatments. While the lowest (-1.7%) was from Dan-eka at T_2 treatment.

The average shoot lengths of the germinated seedlings of all the receptor crops are shown in Table 2. Statistically significant effect (p=0.05) was found at T_5 treatment followed by T_4 and T_3 treatment in all cases and complete inhibition (-100%) of shoot development was occurred in Yaro da kokari and Dan-eka varieties at T_4 and T_5 treatments. Among the survivors, the highest inhibitory effect (-90.2%) was found on Dan'ila at T_5 treatment then followed by Yaro da kokari with (-73.8%) at T_3 treatment while the lowest was on Kanannado with (-0.8%) at T_1 treatment.

Root development was completely inhibited (-100%) in Yaro da kokari and Dan-eka varieties at T_5 and T_4 treatments. Among the survivors, the highest inhibitory effect (-99.7%) was found in Dan-eka at T_3 treatment followed by (-96.7%) on Dan'ila, (-94.3%) on Yaro da kokari at T_5 and T_3 treatments respectively. Minimum inhibitory effects of (-38.0%) was found in Yaro da kokari at T_1 treatment (Table 3).

Complete inhibition (-100%) of lateral root development was found in Yaro da kokari and Dan-eka varieties at T_3, T_4 and T_5 treatments (Table 4). Among the survivors, the highest inhibitory effect on lateral root development was recorded from Yaro da kokari (-96.3%) at T_3 treatment followed by Dan'ila (-88.5%) and Kanannado (-86.6%) at T_5 and T_4 treatments respectively. The lowest inhibitory effects was observed in Aloka variety with (-21.7%) at T_1 treatment.

The experiment revealed that different concentrations of leaf extract inhibited the germination of the seeds to a certain extent which in some cases found to cause complete inhibition of germination of the seeds. Overall growth rate of seedlings was also reduced in almost all the treatments compared with control. So, it was observed that the inhibition of seed germination and seedling growth is dependent on the concentration i.e. inhibition was more as the concentration increased. These findings coincided with the reports of Einhelling (1996), Daniel (1999), Lawan *et al*. (2010), Sazada *et al*. (2009) who reported that allelopathy is concentration dependent phenomenon. Mortality of the seedlings and reduced vigor under laboratory conditions indicated the accumulation of toxic substances (Allelopathic potentials) of the donor plants is harmful to the growth of seedlings of receptor plants.

These findings correlated with the reports of Chou, (1992), Rafiqulhoque *et al*., (2003), Ashrafi *et al*., (2007), Lawan, (2011) reported that many weeds and some plant species contain secondary plant products that have allelopathic potentials which resulted to reduction in germination and growth of seedlings. Response of the bioassay species to the aqueous extracts varied among the five species. Considering the overall treatment among the five bioassay species the Aloka variety was the least sensitive to the aqueous extract followed by Kanannado and Dan'ila while Dan-eka and Yaro da kokari were the most sensitive. Marked reduction in root length was noticed in most of the seedlings compared to shoot length and germination. This is in agreement with the findings of Swami Rao and Reddy (1984) who found the inhibitory effect of leaf extract of *Eucaluptus* (hybrid) on the germination of certain food crops. Zackrison and Nilsson (1992), Chon *et al*. (2002), Jayakumar and Manikandan (2008) have all reported the higher sensitivity of root growth than seed germination.

Table 1: Germination percentage of receptor cowpea varieties to distilled water (T_0) and different concentrations of *A. indica* leaf extracts (T_1-T_5).

Treatments	Dan'ila	Yaro da kokari	Kanannado	Aloka	Dan-eka
T_0	70.0^{bc}	83.3^{a}	98.3^{a}	98.3^{a}	100.0^{a}
T_1	90.0^{a}; (-28.6)	85.0^{a}; (-2.0)	83.3^{ab}; (-15.3)	97.7^{a}; (-1.7)	96.7^{a}; (-3.3)
T_2	83.3^{ab}; (-19.0)	85.0^{a}; (-2.0)	70.0^{bc}; (-28.8)	97.7^{a}; (-1.7)	98.3^{a}; (-1.7)
T_3	75.0^{abc}; (-7.1)	88.3^{a}; (-6.0)	73.3^{bc}; (-25.4)	91.7^{a}; (-6.8)	56.7^{b}; (-43.3)
T_4	75.0^{abc}; (-7.1)	31.7^{b}; (-62.0)	25.0^{d}; (-74.6)	90.0^{ab}; (-8.5)	21.7^{c}; (-78.3)
T_5	65.0^{c}; (-7.1)	5.0^{c}; (-98.0)	56.7^{c}; (-42.4)	83.3^{b}; (-15.3)	1.7^{d}; (-98.3)

Values in the columns followed by same letter(s) are not significantly different P=0.05 according to (DMRT).
Values in the parenthesis indicate the inhibitory effects in comparison to control treatment.

Table 2: Shoot length (cm) of receptor cowpea varieties to distilled water (T_0) and different concentrations of *A. indica* leaf extracts (T_1-T_5).

Treatments	Dan'ila	Yaro da kokari	Kanannado	Aloka	Dan-eka
T_0	7.3^{b}	6.7^{b}	17.4^{a}	17.3^{a}	3.4^{a}
T_1	9.8^{a}; (-34.1)	7.2^{b}; (-7.3)	17.2^{a}; (-0.8)	17.2^{a}; (-1.0)	3.4^{a}; (-1.8)
T_2	9.0^{ab}; (-18.2)	9.2^{a}; (-36.9)	18.3^{a}; (-5.2)	15.4^{b}; (-11.3)	2.9^{a}; (-13.4)
T_3	3.6^{c}; (-50.1)	1.8^{c}; (-73.8)	15.8^{a}; (-9.3)	12.3^{c}; (-28.7)	2.9^{a}; (-13.4)
T_4	2.0^{cd}; (-73.2)	0.0^{d}; (-100)	6.5^{b}; (-62.8)	8.1^{d}; (-53.4)	0.0^{b}; (-100)
T_5	0.7^{d}; (-90.2)	0.0^{d}; (-100)	6.8^{b}; (-61.0)	6.2^{d}; (-61.9)	0.0^{b}; (-100)

Values in the columns followed by same letter(s) are not significantly different P=0.05 according to (DMRT).
Values in the parenthesis indicate the inhibitory effects in comparison to control treatment.

Table 3: Root elongation (cm) of receptor cowpea varieties to distilled water (T_0) and different concentrations of *A. indica* leaf extracts (T_1-T_5).

Treatments	Dan'ila	Yaro da kokari	Kanannado	Aloka	Dan-eka
T_0	7.5^{a}	19.2^{a}	$^{16.2a}$	7.6^{a}	8.6^{a}
T_1	8.8^{a}; (-17.8)	11.9^{b}; (-38.0)	4.9^{b}; (-69.7)	4.6^{b}; (-39.6)	4.6^{b}; (-47.0)
T_2	3.0^{b}; (-60.0)	10.3^{b}; (-46.3)	4.7^{b}; (-70.9)	3.2^{c}; (-57.9)	0.9^{c}; (-89.2)
T_3	1.5^{b}; (-79.6)	1.1^{c}; (-94.3)	4.4^{b}; (-72.9)	1.0^{d}; (-87.2)	0.2^{d}; (-99.7)
T_4	0.5^{b}; (-93.4)	0.0^{c}; (-100)	1.1^{c}; (-93.5)	0.5^{d}; (-94.1)	0.0^{d}; (-100)
T_5	0.3^{b}; (-96.7)	0.0^{c}; (-100)	1.3^{c}; (-92.2)	0.3^{d}; (-95.9)	0.0^{d}; (-100)

Values in the columns followed by same letter(s) are not significantly different P=0.05 according to (DMRT).
Values in the parenthesis indicate the inhibitory effects in comparison to control treatment.

Table 4: Number of lateral root development of receptor cowpea varieties to distilled water (T_0) and different concentrations of *A. indica* leaf extracts (T_1-T_5).

Treatments	Dan'ila	Yaro da kokari	Kanannado	Aloka	Dan-eka
T_0	16.3^{ab}	40.1^{a}	41.9^{a}	14.7^{a}	7.2^{a}
T_1	24.9^{a}; (-48.7)	22.5^{b}; (-43.9)	18.5^{b}; (-55.9)	11.5^{b}; (-21.7)	4.9^{b}; (-32.4)
T_2	11.3bc; (-32.3)	20.5^{b}; (-48.8)	15.6^{b}; (-62.7)	8.2^{c}; (-44.3)	1.9^{c}; (-74.0)
T_3	8.7^{bc}; (-47.8)	1.5^{c}; (-96.3)	16.2^{b}; (-61.3)	6.5^{cd}; (-56.1)	0.0^{d}; (-100)
T_4	4.4^{c}; (-73.7)	0.0^{c}; (-100)	5.6^{c}; (-86.6)	5.2^{cd}; (-64.7)	0.0^{d}; (-100)
T_5	1.9^{c}; (-88.5)	0.0^{c}; (-100)	9.1^{c}; (-78.3)	4.6^{d}; (-68.8)	0.0^{d}; (-100)

Values in the columns followed by same letter(s) are not significantly different P=0.05 according to (DMRT).
Values in the parenthesis indicate the inhibitory effects in comparison to control treatment.

CONCLUSSION

It may be concluded that the water soluble leachates from the fresh leaves of *A. indica* has the allelopathic potential that reduce the germination as well as suppress the growth and development of some cowpea varieties. So we recommended that long-term field studies must be carried out before incorporating *A. indica* in any agroforestry system.

REFERENCES

Ashrafi, Z., Mashhadi, H. R. and Sadeghi, S. (2007): Allelopathic effects of Barley (*Hordeum vulgare*) on germination and growth of wild varley (*Hordeum spontaneum*). *Pakistan Journal of Weed Science Research,* **13(1-2):** 99-112.

Ashrafi, Z., Mashhadi, H. R., Sadeghi, S., and Alizade, H. M. (2008): Effects allelopathical of sunflower (*Helianthus annuus*) on germination and growth of wild varley (*Hordeum spontaneum*). *Journal of Agricultural Technology,* **4(1):** 219-229.

Bokhari, M. H. and Aslam, K. M. (1985). Neem (*Melia Azadirachta* A. Juss). A useful tree in Northern Nigeria. *Annals of Borno* **II**: 83 – 86.

Daniel, W. G. (1999): *Historical review and Current Models of Forest Succession and Interference.* Florida: CRC Press, 237-251**Pp**

Chon, S. U. Choi, S. K. Jung, S. D. Jang, H. G. Pyo, B. S. and Kims, M. (2002): Effects of alfalfa leaf extract and phenolics allelochemicals on early seedling growth of barnyard grass. *Crop Protection.* **5:** 107-138.

Einhelling, F. A. (1996): Interactions involving allelopathy in cropping systems. *Agronomy Journal,* **88:** 886-893.

Gross, D. and Paritheir, B. (1994): Novel natural substances acting in plant growth regulation. *Journal of Plant Growth Regulation,* **13:** 93-114.

Inderjit, N. and Dakshini, K. M. (1992): Formation 7-O glucoside (ononin),an additional growth inhibitor in soils associated with the weed, *Pluchea lanceolata* (DC). Clarke (Asteraceae). *Journal of Chemical Ecology,* **18:** 713-718.

Inderjirt, I. (1996): Plant phenolies in Allelopathy. *Botanical Review*, **62:** 186 – 202

Jafari, L., Kholdebarin, B and Jafari, E. (2007): Phytotoxic effects of *Chenopodium album* L. water extract on higher plants. *American Journal of Plant Physiology,* **2(3):** 221-226 .

Jayakumar, M. and Manikandan, M. (2008): Allelopathic potentials of *Acacia leucopholea* on germination and growth of groundnut and sorghum. *Allelopathy Journal,* **15:** 123-127.

Kohli, R. K., Batish, D. and Singh, H. P. (1998): Allelopathy and its implications in agroecosystems. *Journal of Crop Production,* **1:** 169-202.

Lawan, S. A., Iortsuun, D. N., Alonge, S. O. and Yahya, S. U. (2010): Evaluation of Allelopathic influence of *Acacia sieberiana* and *Eucalyptus camaldulensis* on maize and cowpea. *Biological and Environmental Science Journal for the Tropics,* **7(2):** 26-30.

Lawan, S. A. (2011): Suppressing effects of *Chenopodium album* L. water extracts on some *Amaranthus* species. *Biological and Environmental Science Journal for the Tropics,* **8(1):** 23-26.

Liebman, M. and Ohno, T. (1997): Crop rotation and legume residue effects on weed emergence and growth: applications for weed management. In: Hatfield, J. L. *et al.,* 1997. *Integrated Weed and Soil Management,* Ann Arbor Press, Chelsea, MI, 181-221 **Pp.**

Mabberley, D. J. (1995): *Azadiracta.* 337-343 **Pp.** In: *Flora Malesiana* ser. Spermatophyta. **12(1):** 1-407.

Mukhtar. F. B. (2003): *Introduction to Biostatistics*. 1st ed. Samrid Publishers, Nigeria **Pp** 152.

National Research Council, (1992): *Neem: a tree for solving global problems.* National Academy Press, Washington, DC. 115 **Pp.**

Nwoekeabia, O. D. (1994): *Annual Reports*, Federal Department of Forestry, Federal Ministry of Agriculture, Abuja, Nigeria. 45 **Pp.**

Olofin, E. A. (1987): Some aspect of physical geography of Kano region and related human responses. Unpublished Departmental Seminer Series. Bayero University, Kano.

Rafiqulhoque, A. T. M., Romel, A., Mohammad, B. U. and Hossain, M. K. (2003): Inhibition of germination and growth behavior of some agricultural crops through application of *Albizia saman* leaf water extracts. *Pakistan Journal of Biological Sciences,* **6(7):** 707- 711.

Rice, E. L. (1984): Allelopathy, 2nd Edition. Academic Press, London, 309-316 **Pp.**

Sankaram, A. V. B. (1987): Chemistry, biological activity and utilization aspects of some promising neem extractives. In: Proceedings of the Third International Neem Comference, Nairobi, Kenya, 10th July, 1986. 127-148 **Pp.**

Sazada, S., Ruchi, Y., Kavita, Y., Feroze, A. W., Mukesh, K. M., Sudarshana, S. and Farah, J. (2009): Allelopathic potentialities of different concentration of aqueous leaf extracts of some arable trees on germination and radicle growth of *Cicer arietinum* Var.-C-235. *Global Journal of Molecular Sciences*, **4(2):** 91-95.

Seligler, D. S. (1996): Chemistry and mechanism of allelopathic interactions. *Agronomy Journal,* **88:** 876-885.

Sundra, M. P. and Pote, K. B. (1978): The allelopathic potentials of root exudates from different ages of *Celosia argenta* Linn. *Natural Academic Science Journal,* **1(2):** 56-58.

Swami-Rao, N. and Reddy, P. C. (1984): Studies on the inhibitory effect of *Eucalyptus* (hybrid) leaf extracts on the germination of certain food crops. *Indian Forester,* **110(2):** 218-222.

Von Maydell, H. J. (1986): *Trees and shrubs of the Sahel, their characteristics and uses*. Eschborn Germany. 173-175 **Pp.**

Zackrison, O. and Nilsson, M. C. (1992): Allelopathic effects by *Emperum hermaphroditum* on seed germination of two boreal tree species. *Canadian Journal of Forest Reseach,* **22:** 44-56.

Zoheir, Y. A., Aptin, R., Sedigheh, S., Hassan, M. A. and Mashhadi, H. R. (2008): Study of the allelopathic potential of *Azadiracta indica* (Neem). *Journal of Biological Sciences,* **8(3):** 57-61.

Zoheir, Y. A., Sedigheh, S., Hassan, M. A. Mashhadi, H. R. and Ebrahim, R. M. (2009): Study of bioassay of the allelopathical effect of neem (*Azadiracta indica)* n-hexane, acetone and water soluble extract on six weeds. *International Journal of Biology,* **1(1):** 71-77.