ORIGINAL ARTICLE

In vitro Nematicidal (Anthelmintic) Property of the Seed Extracts of *Anamirta cocculus* (Linn.) Against *Pheretima posthuma* (L. Vaill.)

Umer Qadir and Paul, V.I.*
Department of Zoology, Annamalai University, Annamalainagar 608 002, Tamil Nadu, India

Corresponding author: Dr. V.I. Paul, Associate Professor, Department of Zoology , Annamalai University, Annamalainagar 608 002, Tamil Nadu, INDIA,
Tel: +91 9443046081, E-mail: drissacpaul@gmail.com

ABSTRACT

Anamirta cocculus is a wild climber having antibacterial and piscicidal properties. It is used in Ayurvedic and folk medicines. The primary objective of the present study was to evaluate the in vitro nematicidal or nematodicidal (anthelmintic) activity of methanol, chloroform and petroleum ether extracts of the seeds of *A. cocculus* using the earthworm *Pheretima posthuma* as an experimental model. After calculating the ½ an hour LC_{50s} of all the extracts, the time taken for paralysis as well as death of the worms in each of the extracts was tested using three concentrations each viz.,10, 15 and 20 mg mL^{-1}. All the three extracts produced significant anthelmintic activities in a dose dependent manner. The nematicidal activities of the extracts were either significantly higher or more or less equal to the reference drug Albendazole. Highest nematicidal activity was shown by the methanol extract (20 mg mL^{-1}) with the paralysis and death time of 3±0.3 and 6±0.2 minutes respectively. Moreover, methanol extract exhibited an increased paralytic as well as nematicidal effect over the reference drug Albendazole at all the given experimental concentrations. The results indicated the strong probability of the *A. cocculus* seeds as the potential raw material for developing an effective nematicide that may be highly useful in controlling the plant parasitic nematodes.

***Keywords*:** Albendazole, *Anamirta cocculus*, Anthelmint, earthworm, nematicide, *Pheretima posthuma*

INTRODUCTION

Plants and plant products are widely used in the traditional and folk medicines throughout the world. Similarly, plants form the basis of some important drugs that have been frequently used in Ayurveda, Siddha and Unani system of medicines. Many of these plants are known to have antioxidant, antimicrobial, anti-inflammatory and nematicidal or nematodicidal, (antihelmintic) properties. Parasitic nematodes are of serious concern not only to the animal world but also to many of the economically important plant species (Ellis *et al*., 2008).

Majority of the nematode (helminth) infections are generally limited to tropical regions and are prevalent particularly in third world countries of tropical regions due to poor helminth management practices and can pose large economic and health problems (Dhar *et al*., 1982; Agrahari *et al*., 2011). Chemical control of nematodes coupled with improved crop management has been the important worm control strategy especially for plant parasitic nematodes throughout the world. However, increasing problems of development of resistance in nematodes including helminths (Geert and Dorny, 1995; Coles, 1997) against anthelmintics have led to the proposal of screening phytochemicals for their control. In traditional systems of medicine also, various indigenous plants and plant parts are used as antihelmintics. *Anamirta cocculus* (Linn.) is a wild climber that has been exploited variously by human beings for different applications including Ayurvedic and folk medicines (Satya and Paridhavi, 2011). It has been suggested to possess anti-inflammatory, anti-fungal, anti-microbial, insecticidal, germicidal, antioxidant and anthelmintic properties (Satya and Paridhavi, 2012). In the historical documents, the plant was used in the treatment of bronchitis, chronic skin diseases, foul ulcers, dermatophytosis, phthisis, inflammation, vertigo, flatulence, epilepsy, chorea, paralysis of pharynx, leg and respiratory centre, breast cancer and mastitis (Krishnaraju *et al*., 2006; Mati and de Boer, 2011; Satya and Paridhvi, 2012).

Due to the easy availability, handling convenience as well as anatomical and physiological resemblance of earthworms with nematodes, the former have been widely utilized for evaluating nematicidal (anthelmintic) activity (Sollamann, 1918; Dabadi *et al*., 2011; Ilango *et al*., 2011; Preeti *et al*., 2012; Elias *et al*., 2013). Nematode worms, feed on organic matter, bacteria, insects and plants. Approximately 10% of all nematodes feed on plants and live around or in the roots. The most well known form is the root knot nematode (*Meloidogyne* spp.). This is mainly because of the distinctive galls it causes on infected roots, its wide distribution, and the wide range of plants that it attacks (e.g., common vegetables, ornamentals, fruit tree etc.) (Bridge *et al*., 1990). One of the major crops suffering from nematode attack is banana (*Musa* spp.) and the common plant parasitic nematodes attacking banana are *Radopholus similis, Pratylenchus* spp., *Helicotylenchus multicinctus* and *Meloidogyne* spp. (Gowen and Queneherve, 1990). Foliar feeder (*Aphelenchoides* spp.) is one of the few plant-parasitic nematodes which live in and damage leaves, buds, and other soft tissues of plant parts that are above the ground (Pokharel and Larsen, 2007). Lesion nematodes (*Pratylenchus neglectus* and *Pratylenchus thornei*) reduces yield as much as 70% in susceptible spring wheat varieties with little or no visual symptom expression in the crop canopy, whereas *Pratylenchus vulnus* is the most common root lesion nematode in fruit crops, causing damage in apple, peach, cherry, and grapes (Mai and Mullin, 1975).

In India nematode attack is prevalent in case of many crops such as banana, cotton, tomato etc. While nematode infestation is the basic reason

for low yield of cotton per hectare in India (319 kg lint/hectare) as compared to other countries like Israel (1709 kg lint/hectare) (Gokte-Narkhedkar *et al*., 2006), it causes root and corn disease in banana leading to 20-50% fruit yield loss (Koshy and Sosamma, 2001; Jonathan and Sreenivasan, 2013). In case of tomato, one of the major cause of yield loss in India is root knot nematode (Saravanapriya and Sivakumar, 2005; Kumar and Khanna, 2006). Even though attempts to control plant parasitic nematodes are widespread, biological methods including biopesticides are less common with a few exceptions where antagonistic fungi like *Arthrobotrys dactyloides* have been explored to trap and parasitize plant pathogenic nematodes (Ellis *et al*., 2008). The widespread method of controlling plant nematodes is by applying toxic chemicals such as aldicarb, carbofuran, phorate etc., that could end up with environmental as well as health issues (Gokte-Narkhedkar *et al*., 2006). So there is a need to explore plant origin biopesticides to curb nematode infestation in crop plants. In this context, attempts have been made in the present work to evaluate the nematicidal activity of three solvent extracts of the seeds of the piscicidal plant *A. cocculus* by taking the Indian earthworm *Pheretima posthuma* as an experimental model because anthelmintic/nematicidal studies are usually carried out using *P. posthuma* as an experimental model (Kirtiman, 2012; Majumder, 2013; Chander *et al*., 2014; Das *et al*., 2014).

MATERIALS AND METHODS

Plant material

The seeds of *A. cocculus* belonging to the family Menispermaceae were collected from the wild from Kerala state, India and were dried properly before the commencement of the experiment.

Preparation of seed extracts

Endosperms were collected from the dried seeds of *A. cocculus* and were powdered with the help of an electric grinder. Five hundred grams each of the powdered endosperms were packed in the thimble of the Soxhlet apparatus and were extracted separately with methanol, chloroform and petroleum ether solvents for 72 hrs. Each extract was concentrated using rotary vacuum evaporator and the dried extracts were stored separately for further bioassays by following Qadir *et al*. (2014).

Earthworms

Adult Indian earthworms, *P. posthuma* of nearly equal size (12±1 cm) having anatomical and physiological resemblance with helminths (nematodes) (Vigar, 1984; Shivkar and Kumar, 2003) were collected from moist soil, Bhopal, Madhya Pradesh, India and were brought to the laboratory and used for the experiment.

Estimation of LC_{50}

Prior to the commencement of anthelmintic activity evaluation, static bioassays were conducted to calculate ½ an hour (½h) median lethal concentration (LC_{50}) of the three solvent extracts of the seeds of *A. cocculus* on *P. posthuma* by following APHA *et al*.(1992). Irrespective of their sex, groups of ten adult *P. posthuma* were kept in sterilized Petri plates in triplicate and static bioassays were done with methanol, chloroform and petroleum ether extracts of *A. cocculus* seeds. The exposure concentrations of all the extracts were prepared by dissolving the desired quantity of the respective extract in mg mL^{-1}proportion in 10% propylene glycol prepared in normal saline. Appropriate control groups were also maintained by applying 10% propylene glycol prepared in normal saline without the addition of extracts. The dead worms from each of the Petri plates were removed after the completion of the fixed time interval (½h) and the mortality rates were recorded. The ½h LC_{50} for each of the three extracts was calculated using their percentage of mortality by following Probit method of Finney (1971).

Anthelmintic bioassay

Three different concentrations (10, 15, 20 mg mL^{-1}) each of the three extracts were prepared in 10% propylene glycol in normal saline. Albendazole was prepared by dissolving it in normal saline at concentrations of 10, 15 and 20 mg mL^{-1} to serve as a reference drug. Experimental control consisted of 10% propylene glycol in normal saline and normal control consisted of normal saline only. The earthworms were washed with normal saline to remove all debris, feacal matter etc., and were acclimatized to the laboratory condition before the experimentation.

Anthelmintic activity was assayed following Nargund (1999) and Nayak *et al*. (2009) with some minor modifications using *P. posthuma*. Six earthworms each were placed in separate Petri dishes containing three different concentrations (10, 15, 20 mg mL^{-1}) each of *A. cocculus* extracts (methanol, chloroform and petroleum ether) in triplicate along with standard reference drug (Albendazole), experimental control and normal control. The worms were observed for paralysis and death at room temperature. The mean time for paralysis was noted when there was no movement of any kind by the earthworms except when the worms were shaken / prodding vigorously. Death was confirmed when the worms do not even revive in normal saline. The time of death of worms (min) was recorded after ascertaining that worms neither moved when shaken nor when given external stimuli or normal saline that was followed with fading of the body color, which means their cellular death (Preeti *et al*., 2012). The results from treated groups were compared with reference group, experimental control as well as normal control. Details regarding the composition of the experimental set up are summarized in Table 1.

Statistical analysis

Chi-square (χ^2) test was used to check the heterogeneity of the data used for calculating the LC_{50} values. Results with $P<0.05$ were considered as statistically significant and the 95% fiducial level of upper and lower confidence limits for each of the lethal concentrations was also calculated. The obtained data in each group were compared with the respective reference group using Student's 't' test.

RESULTS AND DISCUSSION

Median lethal concentrations of the three extracts of *A. cocculus* seeds against *P. posthuma* are shown in Table 2. The worms were more sensitive towards methanol extract when compared to other extracts and correspondingly it showed the lowest ½h LC_{50}, LC_{90} and LC_{99} values value. The toxicities of the other two extracts were significantly ($P<0.05$) lesser than that of methanol extract. No mortality was observed in the control groups.

The summary of the nematicidal activity of the seed extracts (methanol, chloroform and petroleum ether) of *A. cocculus* in terms of its anthelmintic effect is given in Table 3. It was observed that all the three concentrations (10, 15, 20 mg mL^{-1}) of methanol, chloroform and petroleum ether extracts of the seeds of *A. cocculus* showed significant anthelmintic activity. In any case, the anthelmintic activity was either significantly higher or more or less equal to the reference drug Albendazole (Table 3).

Table 1. Composition of the experimental setup (in triplicate)

Group	Exposure quantity (mL)	Composition
1	50	Six earthworms + methanolic extract (10 mg mL^{-1})
2	50	Six earthworms + methanolic extract (15 mg mL^{-1})
3	50	Six earthworms + methanolic extract (20 mg mL^{-1})
4	50	Six earthworms + chloroform extract (10 mg mL^{-1})
5	50	Six earthworms + chloroform extract (15 mg mL^{-1})
6	50	Six earthworms + chloroform extract (20 mg mL^{-1})
7	50	Six earthworms + petroleum ether extract (10 mg mL^{-1})
8	50	Six earthworms + petroleum ether extract (15 mg mL^{-1})
9	50	Six earthworms + petroleum ether extract (20 mg mL^{-1})
10 (Experimental control)	50	Six earthworms + 10% propylene glycol in normal saline
11 (Normal control)	50	Six earthworms + normal saline
12 (Reference)	50	Six earthworms + Albendazole in normal saline (10 mg mL^{-1})
13 (Reference)	50	Six earthworms + Albendazole in normal saline (15 mg mL^{-1})
14 (Reference)	50	Six earthworms + Albendazole in normal saline (20 mg mL^{-1})

All the extracts produced significant anthelmintic activity in a dose dependent manner. While methanolic extract showed minimum time needed for paralysis and death of the earthworms, petroleum ether extracts showed maximum time needed for paralysis and death (Table 3). Methanol extract at all the three concentrations (10, 15, 20 mg mL^{-1}) was more effective than the other two extracts as well as reference drug in their respective concentrations. Highest anthelmintic activity was shown by methanol extract at 20 mg mL^{-1} with the paralysis and death time of 3±0.3 and 6±0.2 min respectively. Methanol extract exhibited an increased paralytic as well as anthelmintic effect over the reference drug Albendazole at the given experimental concentrations (Table 3). The potency of the extract was found to be inversely proportional to the time taken for paralysis and death of worms.

Table 2. 1/2h LC_{50}, LC_{90} and LC_{99} values of seed extracts of *A. cocculus* against *P. posthuma*

LC values	Methanol Extract	Chloroform extract	Petroleum ether extract	Albendazole
LC_{50}	7.587	8.144	8.289	8.048
Lower limit	6.889	6.929	6.227	6.758
Upper limit	7.813	8.401	9.241	8.594
χ^2	1.323*	1.997*	2.538*	0.607*
Slope	1.058	1.057	1.120	1.153
LC_{90}	8.162	8.743	9.011	9.667
Lower limit	7.943	8.495	8.557	9.102
Upper limit	8.711	9.618	10.557	10.991
χ^2	1.323*	1.997*	2.538*	0.607*
Slope	1.061	1.059	1.132	1.172
LC_{99}	8.664	9.264	10.147	11.224
Lower limit	8.322	8.884	9.342	10.220
Upper limit	10.203	12.124	15.334	15.251
χ^2	1.323*	1.997*	2.538*	0.607*
Slope	1.068	1.065	1.160	1.217

*Significant at P<0.05.

Another important observation was the progression in the toughness and rigidity of the cuticular layer of the paralyzed worms that were exposed to the extracts. In comparison to the control ones, the cuticular layer of the treated worms became tough and rigid as the time elapsed. The progression of toughness and rigidity of the cuticular layer intensified as the death of the earthworms approached and could be felt by touching the dead worms. The rate of mortality caused by *A. cocculus* seed extracts points to the relationship between percentage of mortality and concentration of the extracts. The significant χ^2 values (Table 2) indicate that mortality rates are not significantly heterogeneous and variables such as individual variations do not significantly affect the median lethal concentrations since they lie within 95% confidence limit. The steepness of the slope also indicates a sharp increase in the mortality rate with a small increase in the exposure concentration. Further, on extrapolation of percentage of mortality, a near 100% mortality of the worms could be predicted at the calculated ½h LC_{90} (Table 2) of the seed extracts of *A. cocculus*. Therefore the calculated ½h LC_{99} may be considered as the absolute acute concentration causing 100% mortality in the stipulated exposure time interval. Previous report (Qadir and Paul, 2014) on controlling the yellow stem borer of paddy using aqueous extract of *A.cocculus* also revealed such toxicological characters of *A. cocculus* seeds.

Table 3. Summary of the *in vitro* wormicidal activity (in triplicate) of various extracts of the seeds of *A. cocculus* on *P. posthuma*

Test substances	Group	Concentration (mg mL^{-1})	Time taken for paralysis (min) $\overline{X}$ ±SD	Time taken for death (min) $\overline{X}$ ±SD
Methanol extract	1	10	10±1**	25±1.2**
	2	15	6±0.8**	18±0.2**
	3	20	3±0.3*	6±0.2**
Chloroform extract	4	10	13±0.2*	29±0.6**
	5	15	8±0.3*	20±1.1**
	6	20	$4±0.5^{NS}$	8±0.4*
Petroleum ether extract	7	10	$15±0.3^{NS}$	31±0.4*
	8	15	$10±1^{NS}$	23±0.3*
	9	20	$5±0.6^{NS}$	$9±0.5^{NS}$
Experimental control (10% propylene glycol in normal saline)	10	–	–	–
Normal control (normal saline)	11	–	–	–
Albendazole (reference)	12	10	16±1.2	35±0.8
	13	15	10±1.3	26±0.5
	14	20	5±1	10±0.2

Based on Student's 't' test; n=18 in each group; comparison made with the respective reference group; NS= not significant; **p<0.01; *p<0.05.

In this context, on the basis of the results of lethal concentrations assays (Table 2) as well as previous reports (Preeti *et al.,* 2012; Majumder, 2013; Das *et al.,* 2014), the treatment concentrations for anthelmintic assays (Table 3) were selected in such a way that it corresponds to the lowest concentrations of the commonly available reports and at the same time above the ½h LC_{99}. This could ensure maximum mortality in reasonably minimum time. The three preliminary extracts of the seeds of *A. cocculus* show significantly different durations for paralysis and death of the earthworms at different concentrations (Table 3) indicating significant variations in the toxicological potencies of the extracts. Qadir *et al.* (2014) also observed the variation in the toxicities of seven extracts of *A. cocculus* seeds and reported that methanol extract was the most toxic one to inhibit microbial growth. The present study is also in agreement with the reports of Qadir *et al.* (2014) that methanol extract is the most potent one to kill *P. posthuma*. Irrespective of such variations in the potencies, all the three extracts exhibit significant nematicidal / anthelmintic activities. Methanol extract shows the highest level of nematicidal potency as well as paralytic activity and thereby making it the best candidate for formulation of biopesticide for the control of nematode infestation in plants. Agrahari *et al.* (2011) also observed significant anthelmintic *activity* in the case of methanol extract of *Jussiaea hyssopifolia*. Recently Qadir *et al.* (2014) have observed rich presence of alkaloids, saponins, phenolic compounds and flavonoids in the methanolic extract of *A. cocculus*. They have also reported that while alkaloids are present in the petroleum ether extract, chloroform extract possesses saponins and phenolic compounds. Many of these components are known to have antiseptic and antimicrobial activities. While alkaloids and phenolic compounds are reported to have sedative as well as anthelmintic

properties, saponins are well known antimicrobial and antiseptic agents (Tiwari *et al*., 2011; Visweswari *et al*., 2013). Saponins also function as molluscicidal as well as anthelmintic agents (Wang *et al*., 2010; Tiwari *et al*., 2011; Visweswari *et al*., 2013). The combined action of the various bioactive compounds present in the methanolic extract in the *A. cocculus* seeds might be responsible for its prominent nematicidal action than the other two extracts. According to Puupponen-pimia *et al.* (2001) and Doughari (2012); alkaloids and phenolic compounds present in plants have an inherent defensive function against herbivores and pathogens.

In fact, the present study indicates that the methanol extract of *A. cocculus* is more effective than the reference drug Albendazole in its anthelmintic activity, making it a potential source of biopesticide to effectively manage the nematode parasites of plants. Previous reports indicate that the size of the organism in many cases is directly related to the concentration of the toxicant needed to kill it and therefore anthelmintic activities are generally demonstrated by finding out a particular concentration of the extract that can kill *P. posthuma* in a given interval of time based on the concept that the same concentration of the extract shall wipe out the tiny helminthes/ nematodes (Bachaya *et al*., 2009; Agrahari *et al*., 2011; Preeti *et al*., 2012). Therefore, nematode parasites of plants being comparatively very small organisms when compared to the experimental organism (*P. posthuma*), a concentration of the extract that cause mortality in *P. posthuma* could cause death of the tiny nematode parasites of plants if properly exposed to it. This could be attained by treating the concerned plant/plant part (e.g., rhizome of banana before planting) with the extract.

The body surface of nematode is a collagen rich extra cellular matrix forming the protective cuticle of the organism and is critical for its viability (Page and Winter, 2003). The phenolic compounds are shown to bind with the glycoprotein on the cuticle of the parasite (Thompson and Geary, 1995) or interfere with energy generation process in helminth parasites by uncoupling oxidative phosphorylation (Martin, 1997) and cause the death of the parasite. In leather industry, plant origin phenolic compounds are commonly used in the tanning operation that imparts structural alteration and stability to collagen of skin matrix through its reactivity and hence make the collagen molecule to aggregate into fibers. Such aggregations results in the loss of flexibility in the collagen matrix (Vidyadhar *et al*., 2010). The possibility of such a chemical interaction between the nematode cuticle and the phenolic compounds of *A. cocculus* extract also cannot be ruled out. Such an interaction in the nematode parasites could also result in the aggregation as well as loss of flexibility in their cuticular collagen leading to its toughness and thereby affecting various physiological functions. At this juncture, it is important to note the toughness experienced in the cuticular layer of the paralyzed earthworms immediately before the death in the present study. Such morpho-physiological changes might have contributed to the paralysis and death of the exposed worms.

Further, saponins which are present in the methanolic and chloroform extracts of *A. cocculus* (Qadir *et al*., 2014) are also reported to have anthelmintic activity. According to Bachaya *et al*. (2009), lethal effect of saponins against helminths (nematodes) may be due to vacuolization and disintegration of integuments in them. The minimum nematicidal activity observed in the petroleum ether extract (more or less similar to Albendazole) of the present study may be attributed to the absence of phenolic compounds, saponins and flavonoids and presence of alkaloids in it (Qadir *et al*.,

2014) as the latter affect the central nervous system (CNS). According to Tiwari *et al*. (2011), in helminths, alkaloids interfere with protein synthesis, suppress transfer of sucrose as well as glucose and act on CNS causing paralysis and death.

CONCLUSION

All the three extracts of *A. cocculus* seeds (methanol, chloroform and petroleum ether) possess prominent nematicidal activities of different intensities besides its other traditional and pharmacological applications. Out of the three, methanol fraction showed maximum wormicidal potency that was significantly higher than the respective reference drug concentrations indicating its potential for developing a nematicide belonging to the biopesticide group. As the minimum experimental concentration (10 mg mL^{-1}) of methanol extract used in the present study could effectively knock out all the earthworms in a lesser time interval than the reference drug Albendazole, the plant parasitic nematodes that are relatively much smaller than earthworms are expected to be killed at this concentration. In short, methanol extract of *A. cocculus* seeds has proved to have promising nematicidal activity especially in comparison to the standard drug. However, it is also necessary to identify and isolate the possible phytochemical exactly responsible for the wormicidal activity.

Acknowledgement

The authors are thankful to the authorities of Annamalai University for the facilities provided.

REFERENCES

Agrahari, AK, Meher, A, Padhan, AR and Dash, S. 2011. Assessment of anthelmintic activity of *Jussiaea hyssopifolia* G. Don. *Asian J. Plant Sci. Res.* 1: 87-91.

APHA (American public health association), AWWA (American Water Association) and WPCF (Water Pollution Control Federation) 1992. Standard methods for the examination of water and waste water. 19th Edition. Washington, DC.

Bachaya, HA, Iqbal, Z, Khan, MN, Jabbar, A, Gilani, AH and Din, IU. 2009. *In vitro* and *in vivo* anthelmintic activity of *Terminalia arjuna* bark. *Int. J. Agric. Biol*. 11: 273-278.

Bridge, J, Luc, M and Plowright, RA. 1990. Nematode parasites of rice. pp. 69-108. *In* Luc M, Sikora RA and Bridge J. (eds.) Plant parasitic nematodes in tropical and subtropical agriculture, CAB International, Wallingford, UK.

Chander, PA, Sri, HY, Sravanthi NBM and Susmitha, UV. 2014. In vitro anthelmintic activity of *Barleria buxifolia* on Indian adult earthworms and estimation of total flavonoid content. *Asian Pac. J. Trop. Dis*. 4(1): S233-S235.

Coles, GC. 1997. Nematode control practices and anthelmintic resistance on British sheep farms. *Vet. Rec.* 141: 91-93.

Dabadi, P, Koti, BC, Swamy, AHMV and Kala, C. 2011. *In vitro* anthelmintic activity of *Mimusops elengi* bark. *Indian J. Novel Drug Deliv.* 3: 307-310.

Das, A, Karmakar, P, Kibria, MG, Debnath, PC, Islam, MS and Sattar, MM. 2014. Comparative phytochemical screening and *in vitro* evaluation of biological activities between aqueous and ethanolic extract of *Momordica charantia* L. fruits. *British. J. Pharmaceut. Res.* 4(6): 739-750.

Dhar, DN, Sharma, RL and Bansal, GC. 1982. Gastro-intestinal nematodes in sheep in Kashmir. *Vet. Parasitol.* 11: 271-277.

Doughari, JH. 2012. Phytochemicals: Extraction methods, basic structures and mode of action as potential chemotherapeutic agents. pp. 1-33. *In* Rao V. (ed.) Phytochemicals – a global perspective of their role in nutrition and health, In: Tech. Availableat:http: //www.intechopen.com/articles/show/title/phytochemicals-extraction-methods-basic-structures-and-mode-of-actionas-potential-chemotherapeutic.

Elias, A, Ravichandran, S, Karthik, T, Maharajan, T, Satyamala, P and Lingeshwari, D. 2013. Pharmacognostical, phytochemical and anthelmintic activity on leaves of *Solanum nigrum* Linn. *Asian J. Res. Biol. Pharmaceut. Sci.* 1: 1-8.

Ellis, SD, Boehm, MJ and Rhodes, LH. (2008): Nematode diseases of plants. OSU Extension Factsheet - Introduction to Plant Disease Series, PP 401.08. http://ohioline.osu.edu/hyg-fact/3000/index.html.(Accessed on 12th January, 2014).

Finney, DJ. 1971. Probit analysis. 3rd edn. Cambridge University Press, London.

Geert, S and Dorny, P. 1995. Anthelmintic resistance in helminths of animals of man in the tropics. *Bull. Seances Acad. R. Sci. Outre. Mer.* 41: 401-424.

Gokte-Narkhedkar, N, Mukewar, PM, Mayee, CD. 2006. Parasitic nematodes of cotton-farmer's hidden enemy. Technical Bulletin from CICR. Central Institute for Cotton Research, Nagpur, India.

Gowen, S and Queneherve, P. 1990. Nematode parasites of bananas, plantains and Abaca. pp. 431-460. *In* Luc M, Sikora RA, Bridge J. (eds.) Plant parasitic nematodes in tropical and subtropical agriculture, CAB International, Wallingford, UK.

Ilango, K, Arulappa, RX and Subburaju, T. 2011. Anthelmintic activity of *Tephrosia spinosa* (L.f.) Pres. *Int. J. Health Res.* 4: 53-56.

Jonathan, EI and Sreenivasan, N. 2013. Managing nematode infestation in banana. *The Hindu*. September 5: 1-14.

Kirtiman, S. 2012. Comparative study of *Withania somnifera* and *Ocimum sanctum* for anthelmintic activity. *ISCA J. Biological Sci.* 1(1): 74-76.

Koshy, PK and Sosamma, VK. 2001. Nematode diseases of plantation crops and their management. pp. 1–176. *In* Proc. of national congress on centenary of nematology in India-appraisal and future plans. New Delhi, India, 5–7 December 2001, Division of Nematology, Indian Agricultural Research Institute, New Delhi.

Krishnaraju, AV, Rao, TVN, Sundararaju, D, Vanisree, M, Tsay, HS and Subbaraju, GV. 2006. Biological screening of medicinal plants collected from Eastern Ghats of India using *Artemia salina* (Brine shrimp test). *Int. J. Appl. Sci. Eng*. 4: 115-125.

Kumar, S and Khanna, AS. 2006. Effect of neem based products on the root-knot nematode, *Meloidogyne incognita* and growth of tomato. *Nematol. Medit*. 34: 141-146.

Mai, WF and Mullin, P. 1975. *Plant-parasitic nematodes: A pictorial key to genera*, 4th edn. Comstock Publishing Associates, Ithaca, London.

Majumder, P. 2013. Investigation of anthelmintic activity of an ignored plant "*Kyllinga nemoralis*" tuber- a potential hope. *Int. J. Pharm. Bio. Sci.* 4(1): 45–52.

Martin, RJ. 1997. Modes of action of anthelmintic drugs. *Vet. J.* 154: 11-34.

Mati, E and de Boer, H. 2011. Ethnobotany and trade of medicinal plants in the Qaysari Market, Kurdish Autonomous Region, Iraq. *J. Ethnopharmacol*. 133: 490-510.

Nargund, VLG. 1999. Anthelmintic activity of 8-fluoro-9-substituted(1,3) benzothiazolo(5,1-b)-1,3,5-triazoles on *Pheretima posthuma*. *Indian Drugs*. 36: 137-139.

Nayak, BS, Jena, PK, Sahu, NP, Nayak, UK and Patro, KB. 2009. Comparative study of anthelmintic activity between aqueous and ethanolic extract of *Solanum surattense* Linn. *Int. J. Pharm. Pharmaceut. Sci.* 1: 103-107.

Page, AP and Winter, AD. 2003. Enzymes involved in the biogenesis of the nematode cuticle. *Adv. Parasitol*. 53: 85-148.

Pokharel, RR and Larsen, HJ. 2007. The importance and management of phytoparasitic nematodes in western Colorado fruit orchards. *J. Nematol*. 39: 96-99.

Preeti, M, Shweta, P and Shreyas, S. 2012. *In vitro* anthelmintic activity of whole plant of *Ventilago denticulata* (Willd) against *Pheretima posthuma*. *Asian J. Pharm. Clin. Res*. 5: 200-201.

Puupponen-Pimia, R, Nohynek, L, Meier, C, Kahkonen, M, Heinonen, M, Hopia, A and Oksman-Caldentey, KM. 2001. Antimicrobial properties of phenolic compounds from berries. *J. Appl. Microbiol*. 90: 494-507.

Qadir, U and Paul, VI. 2014. Evaluation of acute toxicity of the aqueous extract of seeds of *Anamirta cocculus* (Linn.) as a biopesticide against the yellow stem borer (*Scirpophaga incertulas* Walker) of paddy. *Continental J. Biological Sciences* 7 (1): 30 - 39, 2014.

Qadir, U, Paul, VI and Ganesh, P. 2014. Preliminary phytochemical screening and *in vitro* antibacterial activity of *Anamirta cocculus* (Linn.) seeds. *J. King Saud Univ-Sci.* (In press) (http: //dx.doi.org/10.1016/j.jksus.2014.04.004).

Saravanapriya, B., Sivakumar, M. 2005. Management of root-knot nematode *Meloidogyne incognita* on tomato with botanicals. *Natural Product Radiance*. 4(3): 158-161.

Satya, V and Paridhvi, M. 2011. Phytochemical screening and physicochemical standardization of fruits of *Anamirta cocculus* L. Wight and Arn. *Int. J. Comprehen. Pharm*. 2: 1-4.

Satya, V and Paridhvi, M. 2012. Ethno-botanical, phytochemical and pharmacological review of *Anamirta cocculus* (Linn.) Wight and Arn. *Int. J. Rev. Life Sci*. 2: 1-6.

Shivkar, YM and Kumar, VL. 2003. Anthelmintic activity of latex of *Calotropis procera*. *Pharm. Biol*. 41: 263-265.

Sollamann, T. 1918. Anthelmintics: their efficacy as tested on earthworm. *J. Pharmacol. Exp. Ther*. 12: 129-170.

Thompson, DP and Geary, TG. 1995. The structure and function of helminth surfaces. pp. 203-232. *In* Marr JJ. (ed.) Biochemistry and molecular biology of parasites, Academic Press, London, UK.

Tiwari, P, Kumar, B, Kaur, M, Kaur, G and Kaur, H. 2011. Phytochemical screening and extraction: A review. *Int. Pharm. Sciencia*. 1: 98-106.

Vidyadhar, S, Saidulu, M, Gopal, TK, Chamundeeswari, D, Rao, U and Banji, D. 2010. *In vitro* anthelmintic activity of the whole plant of *Enicostemma littorale* by using various extracts. *Int. J. App. Biol. Pharmaceut. Technol*. 1: 1119-1125.

Vigar, Z. 1984. Atlas of medical parasitology, 2nd edn. P.G. Publishing House, Singapore.

Visweswari, G, Christopher, R and Rajendra, W. 2013. Phytochemical screening of active secondary metabolites present in *Withania somnifera* root: Role in traditional medicine. *Int. J. Pharmaceut. Sci. Res*. 4: 2770-2776.

Wang, GX, Han, J, Zhao, LW, Jiang, DX, Liu, YT and Liu, XL. 2010. Anthelmintic activity of steroidal saponins from *Paris polyphylla*. *Phytomedicine*. 17: 1102-1105.