

Foliotoxicity of *Parthenium Hysterophorus L.* and *Lantana Camera L.* on Earth Worm



Botany

KEYWORDS : Toxicity, earth worm, *Lantana camera*, *Parthenium hysterophorus*, in vitro anti-wormal activity.

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ABSTRACT

This investigation was undertaken to study the anti-wormal and anti vermi composting activities of two widespread weeds such as Lantana camera L and Parthenium hysterophorus L. The work highlighted the significance of anti-wormal and anti vermin composting activities of exotic weeds, which were unknowingly added for preparation of vermi-composit, which in turn reduces the decomposition of litter by death of earthworms. Anti-wormal assay was performed on adult Indian earthworm Pheretima posthuma. The time taken for paralysis and death of worms was found lesser in case of methanol extract followed by aqueous extract. The results were compared with standard drug Albendazole and confirm that all extracts were potential toxicity on earth worms slightly vary from its concentration and combinations. In all the experiment set the working concentrations is varied from 100µg/µl to 500 µg /µl and all extract shown the lethality on earth worms.

INTRODUCTION

Parthenium and *Lantana* have been menacing the weeds in agricultural ecology of the country in general and Bijapur in particular for over decades. However, little or no data of scientific studies have been documented regarding the anti-wormal activities with particularly on earth worm *Pheretima posthuma* species and how its impact on soil fertility of this area. It is imperative to identify species that may have the ability to resist or overcome the challenges of the weed, which is largely reducing the quality and quantity of the biomass and its composition. In addition the soil fertility is also threatening inadvertently by contaminating the useful herbaceous species biomass. In our investigation we are attempted to investigate the anti wormal and anti vermi composting activities of two widespread weeds such as *Lantana camera L* and *Parthenium hysterophorus L*. The study may come up with information that it may help to develop management options capable of controlling the aggressive invasion of *Parthenium* and *Lantana* in the agricultural fields and awareness about its harmful effect to agriculture farm land and vermicomposting locations.

The vermicomposting process is a result of the collective action of the earthworms and microflora living in earthworm intestines. The important feature of vermicompost is to improve the soil structure, increasing the water holding capacity and porosity which facilitate the root respiration and growth (Lee, 1992; Parthasarathi et al., 2008). *Lantana camara L.* is an exotic evergreen shrub which belongs to verbenaceae family. The plant is said to have carminative, antispasmodic and anti-rheumatic uses in traditional medicines. The nematicidal compounds are lantanoside, lantanone, linaroside, camarinic acid have been isolated from this plant (Begum et al., 2000). Camaryolic acid, methylcamaralate, camangeloyl acid has beta-sitosterol, 3-O-beta-D-glucopyranoside, octadecanoic acid, docosanoic acid, palmitic acid, camaric acid and lantanolic acid have also been reported (Begum et al., 2003). The plant has antibacterial and antifungal as well as strong antioxidant activities (Basu, B. and Hazra, B. 2006). *Parthenium hysterophorus L.* is an exotic herb which belong to family Asteraceae compositae was accidentally introduced in India in 1956 through imported food grains (Lonkar et al., 1974). It has become naturalized and is spreading at an alarming rate all over India (Sivakumar et al., 2009) and can adopt any climate very easily. It is harmful to all the living beings; it has nearly destroyed all the useful crops and plants, growing near to it. It is known to cause asthma, bronchitis, dermatitis, and hay fever in man and livestock (Narasimhan et al., 1977). Traditional system of medicine reports the efficacy of several natural plants in eliminating worms (Dwivedi et al., 2009). Though the negative impact is very large

to the plants and many lower organism including worms, it is astonishing that very few research groups are working in this area. Our study focuses on anti-wormal and anti-vermicomposting effect of two widespread species.

MATERIALS AND METHODS

Plant materials: Leaves and roots of *Lantana camera. L, Parthenium hysterophorus. L* were collected from in and around the campus of Karnataka State Women's University Bijapur, Karnataka (India) on March 2014 and same were authenticated by qualified plant taxonomist at the Department of Botany, Karnataka State Women's University Bijapur.

Preparation of extracts: Shade dried and coarsely powdered leaf part about 1g of each *Lantana camera. L, Parthenium hysterophorus. L* were prepared and separately subjected for extraction in Soxhlet apparatus using methanol and hot distilled water by using temperature controlled magnetic stirrer at 70°C for one hour and the obtained extract were further subjected to desiccator using anhydrous calcium carbonates and concentrate were stored at room temperature for further experiments.

Animals: Indian adult earthworms (*Pheretima posthuma*) collected from moist soil of the vermi-compositing unit of Women's Technology Park; Karnataka State Women's University Campus and washed thoroughly with sterile double distilled water in order to remove all the faecal matter. The earthworms of 3-6 cm in length and 0.2-0.3 cm in width were used for all the experimental purposes. Earthworms have been used widely for the primary evaluation of antihelminthic compounds in vitro because of easy availability at above said place (Jain, M.L and Jain, S.R.1972).

Drugs and chemicals: The following drugs and chemicals were used. Drugs: Albendazole (BANDY, Mankind Pharma Ltd., New Delhi), Chemicals: all chemicals required for study were analytical grade and procured from Himedia India Pvt. Ltd, Mumbai.

Anti-helminthic Activity: The anti-worm activity was performed on the adult Indian earthworm *Pheretima posthuma* as per the method of (Ghosh et al., 2005 and Rastogi et al., 2009). Albendazole, the standard reference drug, was diluted with double distil water to obtain 100, 200, 300, 400 and 500µg/µl concentrations and was poured into Petri dishes. Water (aqueous) and Methanol extract of the plants were diluted with sterile double distil water to obtain 0.5µg/µl in various volumes (100, 200, 300,400,500 µl). Sterile double distilled water alone served as the positive control. All these dilutions were poured into the Petri dishes accordingly. Thirty petri dishes of equal size were taken

and carefully numbered. Five earthworms (n=5) of similar sizes (about 5-6 cm) were placed in each petri dish at room temperature. The paralysis time was noted down when no movement of any part could be observed, except when the worms were shaken vigorously. Time of death for worms was recorded after confirming that the worms neither moved when shaken vigorously nor when dipped in slight warm water. The paralysis time and lethal time were recorded in terms of hours.

Micro flora free soils were used to study the anti-earth worm toxicity on sterilized soils, with similar concentration of aqueous and methanol plant extracts with earth worm. Similar test were also subjected with fertile soil, the soils samples were collected just few minutes before of conducting experiment and care were taken to retain the texture, composition of soils. All the experiments are conducted in five replicas.

RESULTS AND DISCUSSION

In the present investigation, aqueous and methanol extracts of

leaves of *Lantana camera*.L, *Parthenium hysterophorus*. L leaves were used to evaluate anthelmintic activity against Indian earthworms *Pheritima posthuma*, results were presented in (Table.1). Methanol and aqueous extracts of these extract showed anti-wormal activities in direct, sterile and field fertile soils at different concentration categorized into 100µg/µl, 200 µg/µl, 300 µg/µl, 400 µg/µl, 500 µg/µl and their anti-earth worm activities as shown in (Table.1).

Table.1Anti-worm activities of crude extracts of Parthenium and Lanthana

Extracts	Final Concentration (0.5µg/µl)	Paralysis Time(hour) [Mean±Standard Deviation]	Death Time(hour) [Mean±Standard Deviation]	Extracts	Final Concentration (0.5µg/µl)	Paralysis Time (hour) [Mean±Standard Deviation]	Death Time(hour) [Mean±Standard Deviation]
ALBENDAZOLE	100	0.00±0.00	0.00±0.00	PMED	100	0.51±0.05	1.45±0.08
	200	0.00±0.00	0.00±0.00		200	0.26±0.08	1.02±0.26
	300	0.00±0.00	0.00±0.00		300	0.42±0.11	1.30±0.09
	400	0.00±0.00	0.00±0.00		400	0.14±0.04	1.28±0.13
	500	0.00±0.00	0.00±0.00		500	1.13±0.05	1.40±0.09
PAED	100	1.24±0.13	1.88±0.29	LMED	100	1.09±0.05	1.27±0.11
	200	1.33±0.15	2.13±0.06		200	0.45±0.08	0.97±0.23
	300	1.23±0.13	2.18±0.05		300	0.37±0.13	1.01±0.24
	400	1.23±0.07	2.35±0.16		400	0.27±0.06	1.35±0.08
	500	1.25±0.11	2.26±0.09		500	0.37±0.14	1.12±0.05
LAED	100	1.19±0.08	2.44±0.08	PMESS	100	0.26±0.06	1.19±0.02
	200	1.30±0.12	2.98±0.24		200	0.36±0.05	1.39±0.06
	300	2.24±0.08	2.49±0.11		300	0.33±0.13	1.27±0.08
	400	1.37±0.07	2.49±0.03		400	0.28±0.07	1.47±0.08
	500	0.33±0.16	2.20±0.04		500	0.24±0.04	1.41±0.11
PAESS	100	2.45±0.07	4.43±0.14	LMESS	100	0.29±0.34	0.17±0.05
	200	2.43±0.05	4.39±0.06		200	0.15±0.05	0.34±0.09
	300	3.12±0.03	5.32±0.14		300	0.25±0.09	0.43±0.09
	400	2.16±0.04	5.10±0.04		400	0.21±0.05	0.44±0.10
	500	2.02±0.26	5.39±0.04		500	0.35±0.10	0.53±0.05
LAESS	100	1.22±0.08	2.37±0.06	PMEFS	100	5.26±0.13	6.31±0.18
	200	1.33±0.06	2.32±0.10		200	4.32±0.09	5.97±0.25
	300	1.71±0.29	2.41±0.08		300	4.40±0.12	6.45±0.08
	400	2.20±0.11	2.40±0.04		400	5.16±0.07	6.27±0.12
	500	2.29±0.04	2.47±0.06		500	5.01±0.27	6.15±0.07
PAEFS	100	2.17±0.05	2.87±0.29	LMEFS	100	2.71±0.33	3.05±0.27
	200	2.16±0.07	2.43±0.10		200	2.33±0.07	2.46±0.08
	300	1.43±0.05	2.22±0.09		300	2.33±0.15	2.41±0.08
	400	1.83±0.31	2.20±0.03		400	2.30±0.11	2.47±0.10
	500	1.43±0.04	2.37±0.06		500	2.27±0.12	3.15±0.11
LAEFS	100	3.63±0.21	5.46±0.08				
	200	3.40±0.09	5.22±0.08				
	300	3.23±0.13	5.30±0.09				
	400	3.52±0.06	5.33±0.13				
	500	3.98±0.25	5.21±0.04				

Among the different combinations of extracts the *Lantana* methanolic extract in sterile soil (LMESS) shown more lethal concentration (100µg/µl) 0.15±0.05 hour (200µg/µl) for paralysis and 0.17±0.05 hour required for complete death at concentration (100µg/µl). (Table 1) .In this experiment time taken for paralysis is more or less similar in all concentration in both *Lantana camera.L*, *Parthenium hysterophorus* on direct apply of aqueous *plant* extract. However, the time taken for death of earth worm is slightly varied with different concentration of aqueous extract of both plants. The rationale may be potency of individual worm to withstand the toxic effect of plant extract. The paralysis and death occurred at opening concentration (100 µg/µl) and repeated with all other concentration which depicted in (Table.1 and Fig.1).The combined effect of aqueous *Parthenium hysterophorus* as compared with *Lantana camera L*. *Parthenium* methanolic extract in fertile soil (PMEFS) required more time in paralysis and death compared to all other combinations, it may be in fertile soils the toxic compounds will be absorbed by soil micro-organism, organic matter and inorganic matter; consequently lethal concentration of extract will be further diluted so the required time for paralysis and death of earth worms more (Table.1 and Fig.1)



Fig.1. Anti-earth worm activity of extracts in direct applies (A-Control; B- Treated worm) and fertile soil (C-Control; D- Treated worm)

Many research groups highlighted the tannins were presented in extract of candidate plants and tannins were shown to produce anthelmintic activities. Chemically tannins are polyphenolic compounds (Martin, 1985). Tannins and Phenolics are known to interfere with the energy generation in helminth parasites by uncoupling oxidative phosphorylation (Athnasiadou et al., 2001) and also bind to free proteins in the gastrointestinal tract of host animal or glycoprotein on the cuticle of the parasite, leading to death. It is possible that tannins and phenolic compounds also having in the extracts of *Parthenium* and *Lantana* leaves may be responsible for the anthelmintic activity in addition to unknown compounds present in the crude extract.

In order to analyse the correlation of different combinations applied on earth worm and their degree of effect by using correlation matrix analysis. *Parthenium* aqueous extract direct and *Parthenium* aqueous extract in sterile soil and *Parthenium* methanolic extract in sterile soil shown positive correlation with 0.62 and 0.92 at 0.1 level of significant respectively but in case of *Parthenium* aqueous extract direct with *Parthenium* aqueous extract in fertile soil have shown negative correlation -0.73 at 0.1 level of significant as presented in (Table.2). The correlation matrix revealed their additive and antagonistic lethal effect on earth worms as presented in (Table.2)

Table.2. Correlation matrix of different combinations of crude extracts and their anti-earthworm activity.

	PAED	PAESS	PAEFS	PMED	PMESS	PMEFS	LAED	LAESS	LAEFS	LMED	LMESS	LMEFS
PAED	1	0.62**	-0.73**	0.18	0.92**	-0.14	-0.37	0.17	-0.48*	0.32	0.87**	-0.10
PAESS		1	-0.71**	0.46*	0.42*	0.54*	-0.71**	0.77**	-0.44	0.01	0.85**	0.02
PAEFS			1	0.23	-0.79**	-0.07	0.05	-0.51*	0.67**	0.19	-0.91**	0.60**
PMED				1	-0.22	0.60**	-0.94**	0.11	0.40*	0.63**	0.09	0.71**
PMESS					1	-0.41*	0.01	0.12	-0.65**	0.06	0.82**	-0.36
PMEFS						1	-0.59**	0.34	0.42*	0.27	0.08	0.04
LAED							1	-0.41*	-0.07	-0.42*	-0.39	-0.64**
LAESS								1	-0.66**	-0.60**	0.59*	0.06
LAEFS									1	0.67**	-0.69**	0.18
LMED										1	-0.05	0.22
LMESS											1	-0.24
LMEFS												1

** Correlation is signified at the 0.01 level

* Correlation is signified at the 0.05 level

It can be concluded that the active constituents responsible for anthelmintic activity present in the aqueous and methanolic extracts of *Parthenium hysterophorus L.* and *Lantana camera L.* leaves. Further revision need to isolate and revealed the active compound contained in the crude extract of them and to establish mechanism(s) of action are required. Literature review

reveals that tannins which are chemically polyphenolic compounds (Bate-Smith et al., 1962) are responsible to produce anthelmintic activity (Niezen et al., 1995; Khadatkar et al., 2008; Ghosha et al., 2007; Mali, 2007). Further study may through the light to identify the exact reason for unusual death of earth worm exposed to these plants extract.

CONCLUSION

The aim study is to investigate the detailed account on anti-worm activity of two exotic species such as, *P. hysterophorus* L. and *L.camara* L. and assess the potential impact on vermicompositing industries and also loss of fertility by soil due to inactive or under toxic natural decomposer (earth worm).The experimental evidence obtained in the laboratory model could provide irrational for the traditional use of this plant as vermicompositing substrate and fencing material in their agricultural field unknowingly affect soil fertility and has potential anthelmintic activity. The plant may be further explored for isolation of the active constituent accountable for anthelmintic activity. The phytochemical analysis of the aqueous and methanolic plant extracts indicated the presence of rich chemical constituents. The effect of anti-earthworms on the ability of *P. hysterophorus* L. and *L.camara* L. shown more effect by direct applied aqueous and methanolic and mixture of both on earth worms because of direct contact which take less time to paralysis and kill. Interestingly if we take all extract with different combination on sterile soil with earth worm more time required to paralysis and kill compared to aqueous extract of them and combination. The anti-worm activity on field application is a rough and tracing their potential antiworm activity is herculean task because, area of agriculture field is wild and unknown compound present

in fertile soils, to overcome the difficulty we designed the experimental model to create micro climate in sterile plastic container with fresh fertile soils with ambient moisture and normal room temperature allowed to earth worm and applied various combinations of plant extracts on them. Interestingly, all the earth worm shown paralysis and death on aqueous, methanolic extracts compared to standard reference drug (Albendazole) with concentration ranges from 100µg/µl to 500 µg/µl and it does not show any anti-worm activity; it implies that performed concentration plant extracts were higher than the standard references drug concentration as they shown the potential anti-earth worm activity. Further research is in progress to identify the actual chemical constituents responsible for the lethality to the earth worms.

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