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PHYTOCHEMICAL SCREENINGAND *IN VITRO* NEMATICIDAL ACTIVITY OF CASTOR AND AMALTAS SEED EXTRACTS

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ABSTRACT

Seed extracts of Castor (*Ricinus communis* L.) and Amaltas (*Cassia fistula* L.) were screened for their nematicidal activity against *Meloidogyne incognita* (Kofoid and White) Chitwood. Aqueous extract of seeds at the concentration of 100, 50, 25 and 20 percent showed 100 percent larval mortality whereas delaying and reduction in egg hatching was also observed. Economic importance of non-edible oil seeds of Castor (*Ricinus communis* L.), Amaltas (*Cassia fistula* L.) is due to the presence of variety of secondary metabolites that are useful in pharmaceutical and chemical industry. Present study reveals the presence of terpenoids, steroids, tannins, proteins, coumarins, saponins, phenols, carbohydrates and flavonoidsin methanolic seed extract of Castor and Amaltas. Phlobatannins was appear to be absent in these seeds. Amaltas had highest concentrations of these bioactive compounds followed by Castor.

KEYWORDS: Non-edible oil seeds, Castor, Amaltas, Root-knot nematode, Phytochemicals.

INTRODUCTION

Plant parasitic nematodes are highly destructive plant pathogens causing great economic losses to agricultural crops worldwide (Luc *et al*., 1990; Sasser, 1990; Sikora and Fernandez, 2005). The effect of *Meloidogyne* spp. is immense because of their wide host range of more than 5000 plant species (Trudgill and Blok, 2001) causing severe economic loss to many agricultural crops including vegetables. *Meloidogyne* spp. generally induces morphological and physiological changes within roots, attacking a variety of crops sown and quality are severely affected with yield also reduced (Sasser, 1980). Plant parasitic nematodes were controlled by chemical nematicides, cultural practices and by growing resistant cultivars. However chemical nematicides have their own repercussions for and mostly had been banned in several countries due to their harmful effects on humans and environment (Oka *et al*., 2000). Various organic waste materials were used for soil amendment from early days such as green manures, vegetable and fruits peels, cereal straws, oil seed cakes, cellulosic wastes, livestock wastes and sewage- sludge (Akhtar and Mahmood, 1996; El-Nagdi and Youssef, 2013; Manju and Meena, 2015; Meyer *et al.* 2016). Biodegradation of oil cakes of castor, neem, linseed, groundnut and mustard effectively controlled populations of *Meloidogyne incognita* (Tiyagi *et al*., 2002). Seeds of different plant species have shown strong nematicidal activities like seeds of *Acacia* sp., *Albizzia lebbak, Cassia* sp., *Sesbania* sp., *Medicago* sp., *Phaseolus* sp., *Pisum* sp., *Pongamia* sp., *Sesbania* sp., and *Trigonella* sp. (Khurma and Kumari, 1996; Khurma and Singh, 1997; Khurma and Chaudhry, 1999). Non edible oil seeds like Castor *Ricinus communis* L., and *Cassia fistula* Linn. widely used for medicinal and pesticidal purposes worldwide. Castor (*Ricinus communis* L.) belongs to family Euphorbiaceae. Castor oil contains a wide range of chemical compounds steroids, saponins, alkaloids, glycosides and flavonoid. Traditionally used as laxative, purgative, fertilizers and as fungicides (Jena and Gupta, 2012). Amaltas (*Cassia fistula* L.) a member of leguminaceae family is widely used medicinal plants. Jothy *et al*. (2012) identified the presence of anthraquinones, flavonoids, saponins, tannins and terpenoids in seeds of *Cassia fistula* and also describe antifungal activity especially against yeast. Phenolic compounds possess anti-microbial properties and the main phenolic subclasses in oil seed products, coumarins, flavonoids, tannins and lignin group of compounds (Shahidi and Naczk, 2004).

The present study was designed to investigate nematicidal activity of Castor and Amaltas against root-knot nematode, *Meloidogyne incognita* (Kofoid & White) Chitwood and phytochemical analysis was performed to check the possible nematicidal compounds in these non-edible oil seeds.

MATERIALS AND METHODS

Amaltas (*Cassia fistula* L.), and Castor seeds (*Ricinus communis* L.) were collected from plants cultivated in the University of Karachi, Karachi, Pakistan.

Preparation of aqueous extract: Ten g seed powder of Amaltas (*Cassia fistula* L.) and Castor (*Ricinus communis* L.) was dissolved in 100mL of sterilized distilled water and left for 24 h. After 24 h, filtered the suspension through Wattman filter paper. The filtered suspension was considered as 100% (stock solution) and from this stock solution 50, 25, 20, 15, 10 and 5% solutions were prepared and used for *In vitro* egg hatching and juvenile mortality tests.

Hatching test: *Meloidogyne incognita* (Kofoid and White) Chitwood, eggs were obtained from the roots of infected egg plants (*Solanum melongena* L.) from the field as was describe earlier by Hussey and Barker, (1973). The eggs collected in distilled water in a beaker and were concentrated. One mL of egg suspension (at 40-50 eggs / mL) from the beaker were poured into cavity blocks containing aqueous extracts of Amaltas and Castor and replicated three times. Cavity blocks with egg suspension only served as control. After 24, 48, 72 and 96 h emerging larvae were counted. Mortality of emerged juveniles was also noted (Khurma and Singh, 1997).

Mortality test: *Meloidogyne incognita* eggs were collected in a beaker containing distilled water and incubated at room temperature (30°C) for 24-48 h. One mL of juvenile suspension (at 40-50 juveniles / mL) was poured into each cavity block containing aqueous extracts of Amaltas and replicates three times. Cavity block with juvenile suspension only served as control. After 24, 48, 72 and 96 h observed the dead or static larvae in the cavity block under a low power stereoscope microscope (Cayrol *et al*., 1989).

Phytochemical analysis: Phytochemical studies were carried to find out the presence of Carbohydrates (H_2SO_4 Test), Tannins ($FeCl_3$ Test), Saponins (Froth Test), Flavonoids (H_2SO_4 Test), Terpenoids (Salkowski Test), Steroids (Salkowski Test), Coumarins (Sodium hydroxide test), Phenol (Lead acetate Test), Protein (H_2SO_4 Test) and Phlobatannins (HCl Test). All chemicals used were of analytical grade (Harborne, 1973; Evans *et al.*,, 2009; Pochapski *et al*., 2011).

RESULTS AND DISCUSSION

Castor aqueous extract was most effective at 100 and 50% concentrations in inhibiting egg hatching and at 5% concentration egg hatching stands at 35% (Fig. 1). In Amaltas complete inhibition of egg hatch occurred with 100% and 50% aqueous extracts and at10% concentration egg hatching was 57.7% after 96 h interval of time. The egg hatching was maximum in lower concentrations (Fig. 1).

Castor aqueous extract was highly effective at all concentrations and showed strong nematicidal activity and juvenile mortality in castor extract was from 100 to 97% (Fig. 2).*Cassia* aqueous extract was effective at all dilutions and at 5% concentration juvenile mortality was 86% (Fig. 2).

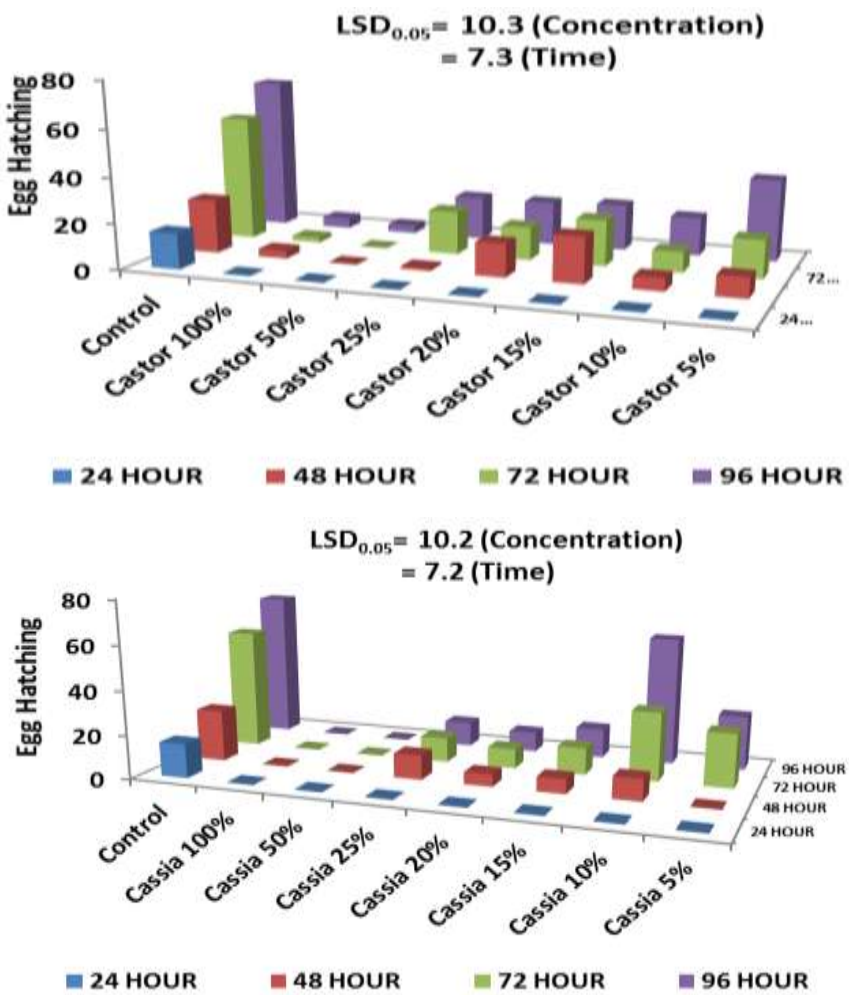

Fig. 1. Effect of seed extracts of Castor (A) and Amaltas (B) on egg hatching of root-knot nematode, *Meloidogyne incognita*.

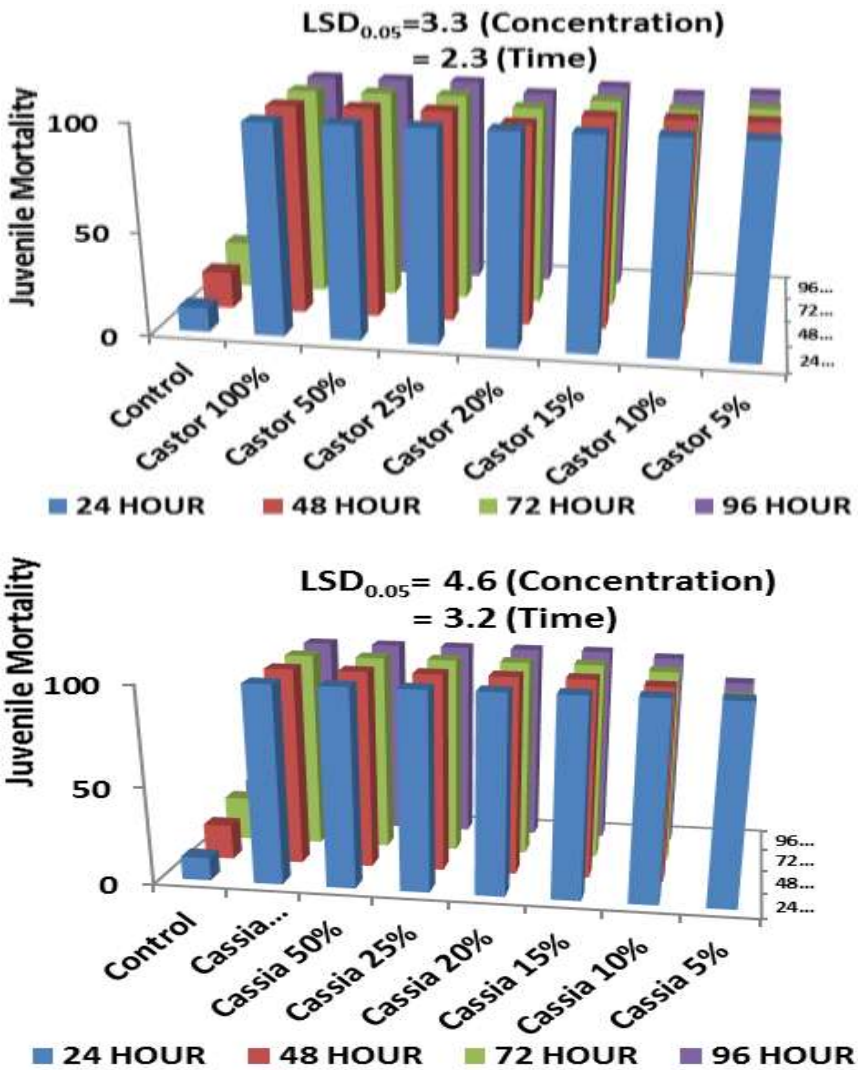

Fig. 2. Effect of seed extracts of Castor (A) and Amaltas (B) on larval mortality of root-knot nematode, *Meloidogyne incognita.*

Biodegradation of oil cakes of castor, neem, linseed, groundnut and mustard effectively controlled populations of *Meloidogyne incognita* (Tiyagi *et al*., 2002). Seeds of different plant species had been shown to possess strong nematicidal activities (Khurma and Kumari, 1996; Khurma and Singh, 1997; Khurma and Chaudhry, 1999). In the present study seeds of Castor and Amaltas were found effective and inhibited egg hatching of root knot nematodes *M. incognita.* In aqueous extract egg hatching was reduced significantly and root knot nematode larval mortality increasing concentrations of aqueous extract had increased its potency. Khurma and Mangotra (2004) reported that high concentrations of seed extracts had high mortality rates and inhibited egg hatching as compared to low concentrations. Khurma and Singh (1997) reported the nematicidal potential of different seed extracts against *M. incognita* and *M. javanica.* Similar results were given by Debanand *et al*. (2000) who used neem leaf extract, neem seed kernel, *Melastomama labathricum* leaf, *Polygonum hydropiper* leaf, *Ageratum conyzoides* leaf extracts and concluded that all extracts were toxic to juveniles and their efficacy increases in higher concentrations of extracts and time of exposure.

The Phytochemical screening of Castor, and Amaltas showed the presence of terpenoids, steroid, tannin, protein, coumarins, saponins, phenol, carbohydrate and flavonoids in the methanol residue of these seed extracts while phlobatannins were absent in both seed extracts (Table 1). Concentration of terpenoid was highest in Amaltas as compared castor seed extracts. Steroids were present in both seed extracts. Tannin was detected in Amaltas while absent in Castor. Coumarins was present in both seed extracts. Saponins was absent in both seed extracts. Phenols were detected in high quantity in Amaltas and Castor as indicated by presence of large amount of white precipitates in the test tubes. Carbohydrate was detected from both seed extracts but its quantity is more in Castor seeds as indicated by appearance of dark violet rings in their test tubes. Flavonoids were abundant in Amaltas seed with dark yellow color formation in the test tube. Protein was completely absent in Amaltas and Castor. The constituents such as alkaloids, carbohydrates, tannins, flavonoids, have curative activity against various ailments including certain pathogenic organisms which justifies its use as a traditional medicine.

Table 1. Phytochemical screening of seed extracts of Castor and Amaltas.

Compounds / Test	Indications	Castor	Amaltas
Terpenoids (Salkowski Test)	A reddish brown colouration at the interface indicates the presence of terpenoids	++	+++
Steroids (Salkowski Test)	Presence of reddish brown ring at the junction of two liquids indicates presence of steroids	+++	+++
Tannins ($FeCl_3$ Test)	Presence of dark green coloration indicated the presence of tannin	−	++
Coumarins (NaOH Test)	Presence of yellow coloration indicates the presence of coumarins in the test tube	++	+++
Saponins (Foam Test)	Froth appearance in the test tube indicates presence of saponins	−	−
Phenols (Lead acetate Test)	Presence of white precipitates in the solution indicates presence of phenol	+++	+++
Carbohydrates (H_2SO_4 Test)	Violet ring formed at the junction of two liquids in test tube indicates the presence of carbohydrates	++	−
Flavonoids (H_2SO_4 Test)	The solution in the test tube will turn yellow in color indicated the presence of flavonoid	+	+++
Proteins (H_2SO_4 Test)	Presence of white precipitates indicates the presence of proteins	−	−
Phlobatannins (HCl Test)	Appearance of red precipitate indicates the presence of phlobatannins	−	−

+ = indicates the presence of phytochemicals; - = indicates the absence of phytochemicals; +++ = shows high concentration; ++ = shows moderate concentration

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