

**IN VITRO EVALUATION OF BIOPRODUCTS ON EGG HATCHING AND  
JUVENILE MORTALITY OF *MELOIDOGYNE INCOGNITA***

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**ABSTRACT**

Effects of bioproducts (Faline, Axiom and Vampire)\*\* were evaluated on mortality of juveniles and egg hatching of *Meloidogyne incognita*. Mortality of *M. incognita* was directly proportional to the concentrations of bioproducts (Faline, Axiom and Vampire) over the time of exposure. The mortality of J<sub>2</sub> was significantly (P = 0.05) affected by all treatments. The 1% concentrations of Faline caused significant (P = 0.05) mortality from 0.5% and 0.25 concentrations at all time intervals. Egg hatching of *M. incognita* was inversely proportional to the concentrations of bioproducts (Faline, Axiom, Vampire) over the time of exposure. The 1% concentrations of Faline caused significant (P = 0.05) reduction in hatching from 0.5% and 0.25 concentrations at all time intervals.

**Keywords:** Hatching, Mortality, Faline, Axiom, Vampire, *Meloidogyne incognita*.

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\*\*\* These are Homoeopathic Natural Bio Pesticides from WS Homoeopathic Pharmacy and Research Centre, Lahore Pakistan.

## INTRODUCTION

Among the plant parasitic nematodes root knot nematodes (RKN) are able to reproduce on over 2000 species of plants (Sasser and Freckman, 1987) and cause approximately 50% damage of overall nematode damage. RKN *Meloidogyne* spp were reported as the most abundant and wide spread nematode (Timm and Ameen, 1960; Ahmad, 1977 and Mian, 1986). Brinjal is affected by *Meloidogyne* spp. And cause the significant damage to the crop (Singh, 1973). It has been reported that *M.incognita* cause 28-47% yield loss in tomato (Patel et al., 1999). The *Meloidogyne* spp affects yield and quality of the crop (Sasser, 1980). The RKN cause major morphological and physiological changes within roots. After hatching from eggs juveniles penetrate in to roots of the host plant and approach the vascular system as RKN is the vascular feeder. The symptoms of nematode infection include the formation of root galls, mineral deficiency, nutrient and water uptake deficiency, growth reduction, wilting and poor yield (Abad *et al.*, 2003).

There are several methods used to control the nematode and cause harmful effect such as deterioration of environment, kill the beneficial organisms and human health hazard (Duncan, 1991) and biodegradable in nature (Tiyagi and Ajaz, 2004). Extracts of plants exhibit biochemical mechanisms to counteract the activity of nematodes. Many plant species belonging to 57 families have been shown to contain nematicidal compounds (Sukul, 1992). The infestation of RKN on vegetable crop reduced by Mycorrhizal fungi has been reported (Hussey and Roncadori, 1982; Vaast, 1997).

Plant parasitic nematodes reduced by the application of soil amendments in all over the world (Khan, 1976; Muller and Gouch, 1982; Rodriguez-Kabana and Morgan-Jones, 1987). The microorganisms decompose the organic material which results in the increased enzymatic activity of amended soil and accumulation ammonia which have nematicidal properties (Rodriguez-Kabana, 1986). Many organic compounds including oil-seed cakes, chopped plant parts and plant extracts have been used as nematode controlling agents (Akhtar and Alam, 1993; Muller and Gooch, 1982; Tiyagi *et al.*, 1988; Tiyagi and Ajaz, 2004). The mode of action of azadirachtin has been studied (Akhtar, 2000; Immaraju, 1998; Mordue and Blackwell, 1993). Nematicidal activity on mortality and egg hatching of second stage juveniles has been tested by the application of *Berberis vulgaris* (Lodhi *et al.*, 2002).

Many chemical pesticides are use to control nematodes but the over dose of chemical pesticides has affected health, ecology, resistance of pest. Although the use of chemical nematicides have been reported as an effective measure to control nematodes but their toxic effect on the environment and particularly on non-target organisms (Akhtar and Malik, 2000; Anastasiadis *et al.*, 2008), promoting the need for new, safe and effective options (Zuckerman and Esnard, 1994) and develop the interest to evaluate the nematicidal compounds in plants (Chitwood, 2002).The most appropriate and safest strategy to control *Meloidogyne* infection is bio control. Therefore this research was conducted to promote the use of bio products and to evaluate the effect of bioproducts such as Faline, Axiom and vampire having the origins of neem and marigold extract, neem extract and *Berberis vulgaris* respectively

on the mortality and egg hatching to evaluate whether these products are nematicidal or nematostatic.

## **MATERIALS AND METHODS**

### **Preparing the concentrations of bioproducts:**

There were three bioproducts (Faline, Axiom and Vampire). There were three concentrations (1%, 0.5% and 0.25%) of each bioproduct as standard “S”. S/2 and S/4.

There were 4 treatments. Each treatment was replicated 5 times. (T1= Faline), (T2= Axiom), (T3= Vampire) and (T4= Control).

### **Extraction of eggs:**

After seven days of transplantation of brinjal (var. Dilnashen) seedlings each pot was infested with 5000 J2 by making holes around the plant rhizosphere to maintain the mass culture of root knot nematodes. Eggs were extracted from galled roots of brinjal using 1% NaOCl solution (Hussey and Barker, 1973). The desired inoculum density was prepared by stirring eggs suspension in tap water.

### **Extraction of juveniles:**

Juveniles were extracted by incubating the egg mass at room temperature (34-38°C).

### **Effect of root extract on egg hatching:**

For hatching test, eggs of *M. incognita* were obtained from the roots of egg plant and collection was done by the method of Hussey and Barker (1973). Eggs suspension was prepared in distilled water and 1 ml suspension containing 50 eggs were poured in petri plate according to the following procedure and petri plates will be kept at room temperature (34-38°C). Petri plates without bioproduct were served as control.

There were 4 treatments. For three bioproducts (Faline, Axiom and Vampire) and each product was applied in three concentrations (1%, 0.5% and 0.25%). Five replicatio ns were done for each concentration. The data was assessed after 24, 48 and 72 hours.

#### **Effect of root extracts on larval mortality:**

For mortality test, freshly hatched second stage juveniles of *M. incognita* was suspended in sterile distilled water and 1 ml of this suspension containing 50 juvenile/ml were placed in each petri plate according to the above mentioned scheme. Petri plate without extract of microbial antagonist and bioproduct were served as control. There were five replicates of each treatment and kept at room temperature (34-38°C). The numbers of juveniles killed after 24, 48 and 72 hours intervals was recorded using a stereoscope. Analysis of Variance was applied on the collected data and means were separated using Duncan Multiple Range Test (Gomez and Gomez, 1984).

## **RESULTS**

#### **Juvenile mortality:**

The mortality of J2 of *M. incognita* was differentially affected by all treatments (Table 1). The J2 treated with Faline had significant ( $P = 0.05$ ) more effect on the mortality than that treated with other two bioproducts including Axiom and Vampire at all time intervals (24, 48 and 72 hours) of application. There is significant ( $P = 0.05$ ) difference on the effectiveness of Faline on the mortality of J2 at all concentrations at all time intervals. The mortality of J2 with Faline was significantly

( $P = 0.05$ ) affected by all concentrations. Faline at its 1% concentration had significant ( $P = 0.05$ ) more effect over all other concentrations after 24, 48 and 72 hours. Faline at its 0.5% concentration caused significant ( $P = 0.05$ ) mortality over 0.25% concentration at all time intervals. Axiom at 100% concentration caused significant ( $P = 0.05$ ) more effect on the mortality over all other concentrations at all time intervals. At 50% concentrations Axiom caused significant ( $P = 0.05$ ) more effect on the mortality over 25% concentration at 72 hours of exposure. Axiom at 25% concentration caused less significant ( $P = 0.05$ ) mortality after all time intervals. Vampire at its 100% concentration caused significant ( $P = 0.05$ ) more effect on the mortality from all other concentrations at all time intervals. At 50% concentration Vampire caused significant ( $P = 0.05$ ) mortality over 25% concentration at 72 hours of exposure. Vampire at 25% concentration caused less significant ( $P = 0.05$ ) mortality at all time intervals.

### **Egg hatching:**

The results revealed that all the treatments varied significantly ( $P = 0.05$ ). Results showed that egg hatching of *M. incognita* was inversely proportional to the concentrations of Faline, Axiom and Vampire. Egg hatching of *M. incognita* was significantly decreased with the duration of exposure (Table 2). There is significant ( $P = 0.05$ ) difference on the effectiveness of Faline on the egg hatching at all concentrations at all time intervals. The egg hatching with Faline was significantly ( $P = 0.05$ ) affected by all concentrations. Faline at its 1% concentration had significant ( $P = 0.05$ ) reduced egg hatching over all other concentrations after 24, 48 and 72 hours. Faline at its 0.5% concentration caused significant ( $P = 0.05$ ) reduced hatching

over 0.25% concentration at all time intervals. Axiom at 100% concentration caused significant ( $P = 0.05$ ) reduced egg hatching over all other concentrations at all time intervals. At 50% concentrations Axiom caused significant ( $P = 0.05$ ) reduced egg hatching over 25% concentration at 72 hours of exposure. Axiom at 25% concentration caused less significant ( $P = 0.05$ ) egg hatching after all time intervals. Vampire at its 100% concentration caused significant ( $P = 0.05$ ) reduced hatching from all other concentrations at all time intervals. At 50% concentration Vampire caused significant ( $P = 0.05$ ) reduced egg hatching over 25% concentration at 72 hours of exposure. Vampire at 25% concentration caused less significant ( $P = 0.05$ ) egg hatching at all time intervals.

## DISCUSSION

The potential of using plant extracts in controlling plant parasitic nematodes has been shown by several authors (Adegbite, 2005; Opareke *et al.*, 2005; Orisajo *et al.*, 2007 and Abbasi *et al.*, 2008;). The inhibitory effect observed in egg hatching according to Adegbite and Adesiyun (2005), might be due to the presence of neem extracts that possesses ovicidal and larvicidal properties. Opabode and Adeboye (2005), showed that Nigeria is endowed with many indigenous plant species, some of which are used for herbal medicine. The use of leaf extract is suggested as a potential substitute for synthetic nematicides used in the management of root knot disease of cacao seedlings (Orisajo *et al.*, 2007). This study has shown that the bioproducts (Faline, Axiom and Vampire) having plant extracts as active ingredient has some nematicidal effects on *M. incognita* in brinjal seedlings. Further studies will be conducted in the field to ascertain the nematicidal ability of the extracts in the soil.

Table 1. Effect of bioproducts on mortality of *Meloidogyne incognita*.

Bioproducts	Doses(%)	Mortality after		
		24hrs	48hrs	72hrs
Faline	1	39.00a	42.40a	43.20a
	0.5	30.80c	30.00d	33.80d
	0.25	21.20e	21.40g	23.00g
Axiom	1	35.40b	38.00b	39.00b
	0.5	25.40d	26.60e	26.60e
	0.25	16.00f	17.20h	17.40h
Vampire	1	27.00d	33.80c	35.80c
	0.5	17.60f	23.40f	25.20f
	0.25	7.80f	14.60i	15.00i
Control		3.00h	4.00j	5.00j
LSD Values		2.31	1.698	1.266

Each data is a mean of five replications.

Table 2. Effect of bioproducts on egg hatching of *Meloidogyne incognita*.

Bioproducts	Doses(%)	Egg hatching after		
		24hrs	48hrs	72hrs
Faline	1	1.6g	3.00g	8.00e
	0.5	9.6e	12.00e	17.20c
	0.25	17.0c	21.00c	27.20b
Axiom	1	2.4fg	4.20g	5.40f
	0.5	11.0de	14.60d	15.60d
	0.25	20.2b	23.20b	26.60b
Vampire	1	3.4f	6.60f	8.20e
	0.5	12.0d	15.40d	16.20cd
	0.25	21.0b	24.60b	27.80b
Control		28.0a	33.60a	47.40a
LSD Values		1.698	1.70	1.570

Each data is a mean of five replications.



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