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Full Length Research Paper

Allelopathic inhibition of seedling emergence in dicotyledonous crops by *Cucumis* bio-nematicide

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Crop yield reduction is proportional to the initial nematode numbers at planting. Thus, nematicidal seed dressing is important in the management of plant-parasitic nematodes. When used as post-emergent bio-nematicide, crude extracts of ground *Cucumis myriocarpus* fruits consistently suppress numbers of the southern root-knot nematode (*Meloidogyne incognita*). This study was initiated to determine suitability of crude extracts of ground *C. myriocarpus* fruits as a pre-emergent bio-nematicide on 10 cultivars of different dicotyledonous crops. In each trial, ten levels of crude extracts of *C. myriocarpus* fruits viz. 0, 0.25, 0.50, 0.75, 1.00, 1.25, 1.50, 1.75, 2.00 and 2.25 g, were arranged in a randomised complete block design (RCBD), with five replicates, under greenhouse conditions. Successful seedling emergence was recorded. Seedling emergence percentage had significant ($P \leq 0.01$) negative quadratic relationship with concentrations of crude extracts, with the concentration of crude extracts of *C. myriocarpus* explaining more than 90% to the total treatment variation in mean seedling emergence of all tested crops. In conclusion, crude extracts of ground *C. myriocarpus* fruits are not suitable for use as pre-emergent bio-nematicide for seed dressing in the 10 tested crops.

Key words: Allelochemicals, allelopathy, auto-allelopathy, concentration-dependent, cucurbitacin A, crude extracts, synthetic nematicides.

INTRODUCTION

Drawbacks of synthetic nematicides and conventional organic amendments on management of plant-parasitic nematodes are well-documented (Kokalis-Burelle and Rodríguez-Kábana, 2006). In an attempt to ameliorate these drawbacks, Mashela (2002) developed the ground leaching technology (GLT), which involves spot application of ground materials from selected plant organs in a shallow hole around the base of the stem at transplanting at 0.20 - 0.71 tonnes ha^{-1} (Mashela, 2002; Mashela and Nthangeni, 2002). This technology relies on irrigation water to leach potent bio-chemicals into the rhizosphere of plants. In a number of screening trials, ground wild cucumber (*Cucucmis myriocarpus*) fruits, castor bean (*Ricinus communis*) fruits and fever tea (*Lippia javanica*) leaves consistently reduced densities of the southern root-knot nematode (*Meloidogyne incognita*) in root and soil samples (Mashela, 2002; Mashela and Nthangeni, 2002; Mashela et al., 2010). Regardless of the organic amendment source, when used as post-planting bio-nematicides, the materials had fertiliser effect and had no effect on soil pH except *L. javanica.* The efficacy of ground *C. myriocarpus* fruits on nematode suppression was comparable to that of synthetic systemic nematicides, namely aldicarb and fenamiphos (Mashela et al., 2008).

Generally, the damage caused by nematodes on plants is directly proportional to the initial population density of nematodes (Pi) at planting (Seinhorst, 1965). Thus, it is preferable that Pi be at its lowest at planting. Most synthetic nematicides have been successful because they could be used to dress seeds without inducing phytotoxicity. Crude extracts of *Cucumis* bio-nematicides at 25-150 g material per litre of water under laboratory conditions inhibited germination of tomato, watermelon and butternut squash (Mafeo and Mashela, 2009). However, these quantities were far above the 0.20

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Table 1. ANOVA of crude extracts of C*ucumis myriocarpus* fruits on seedling emergence of bean, chili, cucumber, eggplant and lettuce (n = 5).

Sources of variation	Degrees of freedom	Bean		Chili		Cucumber		Egg plant		Lettuce	
		SS	Percent (%)	SS	Percent (%)	SS	Percent (%)	SS	Percent (%)	SS	Percent (%)
Rep	4	0.171	2.77 ^{ns}	0.171	2.77^{ns}	0.114	1.71^{ns}	0.114	1.71^{ns}	0.400	5.98^{ns}
Treatment	6	4.971	81***	4.971	81***	5.886	88***	4.686	70***	3.486	52***
Error	24	1.029		1.029		0.686		1.886		2.800	
Total	34	6.171		6.171		6.686		6.686		6.686	

*** Significant at P = 0.01, ns = not significant at P ≤ 0.05.

tonnes ha^{-1}material recommended by the GLT system (Mashela, 2002). The objective of this study was to determine the effect of crude extracts of *C. myriocarpus* fruits on seedling emergence of ten dicotyledonous crops when used within the recommended range of the GLT systems. However, growing mixture in pots was not infested with nematodes.

MATERIALS AND METHODS

Experimental site and growth condition

The study was conducted at the Horticultural Unit of the University of Limpopo (23º53′10″S, 29º44′15″E) in September to December, 2009. Fruits of *C. myriocarpus* were locally collected. Thirty 15-cm-diameter plastic pots were placed on greenhouse benches and filled with 5 L growing mixture, comprising Hygromix and pasteurized sand (3:1 v/v). Ambient day/night temperatures averaged 27/18℃. Each crop was assessed in a separate experiment. Fruits of *C. myriocarpus* were dried at 52℃ for 5 days in air-forced ovens to minimize the loss of volatile phytochemicals (Makkar, 1999). Dried crude extract materials were ground in Wiley mill through 1-mm-mesh sieves. Prior to use, the ground material was stored at room temperature in sealed plastic bags.

Experimental design, treatments, procedures and bioassays

Ten dicotyledonous crops: Bean (*Phaseolus vulgaris*) cv. 'Contendor', chili (*Capsicum frutescens*) cv. 'Long slim cayenne', cucumber (*Cucumis sativas*) cv. 'Delight green F1', eggplant (*Solanum melongena*) cv. 'Black beauty', lettuce (*Latuca sativa*) cv. 'Great lakes', pea (*Pisum sativum*) cv. 'Hygrotech J12082', pepper (*Capsicum annum*) cv. 'Capistrano', sunflower (*Helianthus annaus*) cv. 'PAN 7033', tomato (*Lycopersicon esculentum*) cv. 'Floradade' and watermelon (*Citrullus lanatus*) cv. 'Crimson giant' plants were studied. Ten treatments: 0, 0.25, 0.50, 0.75, 1.00, 1.25, 1.50, 1.75, 2.00 and 2.25 g crude extracts of *C. myriocarpus* fruits/pot, were arranged in a randomised complete block design with five replications. Two seeds/hole were planted at commercially prescribed depths. Crude extracts of *C. myriocarpus* fruits as organic amendments were applied in separate holes at planting, with growing medium irrigated to field capacity. Each pot was irrigated with 250 ml tap-water every other day.

Data collection and analysis

Successful seedling emergence was viewed as the appearance above the soil surface of the hypocotyls, with the exception for pea (Hartman et al., 2002). Data were collected every day for 14 days, expressed as percentage seedling emergence and subjected to one-way analysis of variance (ANOVA) with Statistix 9.0 (Statistix Analytical Software, 1985 - 2008) and means were separated using Duncan multiple-range test (DMRT). When means were different (P ≤ 0.01), lines of the best fit between emergence and concentration of crude extracts of *C. myriocarpus* fruits were generated.

RESULTS

Crude extracts of *C. myriocarpus* fruits significantly (P ≤ 0.01) inhibited emergence of the 10 test cultivars. Partitioning of the sum of squares indicated that the degrees of treatments explained 81, 81, 88, 70 and 52% of the total treatment variation in emergence of bean, chili, cucumber, eggplant and lettuce, respectively (Table 1).

Similarly, treatments explained 81, 88, 79, 54 and 64% of the total treatment variation in emergence of pea, pepper, sunflower, tomato and watermelon, respectively (Table 2).

Emergence of all cultivars tested had strong negative quadratic relationships with the 10 levels of crude extracts of *C. myriocarpus* fruits organic amendment. Treatment levels contributed 96, 99, 99, 99, 96, 95, 99, 99, 99 and 91% to the total treatment variation in mean seedling emergence of bean, chilli, cucumber, eggplant, lettuce, pea, pepper, sunflower, tomato and watermelon, respectively (Figure 1).

DISCUSSION

In all cultivars emergence was reduced by crude extracts of *C. myriocarpus* fruits. However, within the organic amendment range used in this study, when applied as a post-emergent bio-nematicide, crude extracts of this plant consistently reduced *M. incognita* race 2 on tomato with strong fertilizer effect at various conditions (Mashela, 2002; Mashela and Nthangeni, 2002; Mashela et al., 2008). At levels greater than the maximum used in this study, crude extracts of *C. myriocarpus* fruits completely inhibited germination of tomato, watermelon and butternut

Table 2. ANOVA of crude extracts of C*ucumis myriocarpus* fruits on seedling emergence of pea, pepper, sunflower, tomato and watermelon (n = 5).

Sources of variation	Degrees of freedom	Pea		Pepper		Sunflower		Tomato		Watermelon	
		SS	Percent (%)	SS	Percent (%)	SS	Percent (%)	SS	Percent (%)	SS	Percent (%)
Rep	4	0.171	2.77^{ns}	0.114	1.71^{ns}	0.171	3.06^{ns}	0.171	2.17^{ns}	0.171	3.05^{ns}
Treatment	6	4.971	81***	5.886	88***	4.400	79***	4.286	54***	3.600	64***
Error	24	1.029		0.686		1.026		3.429		1.829	
Total	34	6.171		6.686		5.597		7.886		5.600	

*** Significant at P = 0.01, ^{ns} Not Significant.

squash by 91, 97 and 91%, respectively (Mafeo and Mashela, 2009). The suppression of growth of one plant species by another plant species through chemicals is referred to as allelopathy (Inderjit and Duke, 2003). Germination and emergence are chemical and physical processes, respectively (Hartmann et al., 2002). Inhibition of emergence in this study was concentration-dependent. Generally, biological activity of allelochemicals in germination is concentration-dependent with a response threshold being below that where growth is stimulated (Enhellig, 1986).

Most other bio-nematicides have strong inhibition on seedling emergence. Crude extracts of neem (*Azadirachta indica*), widely used as a bio-nematicide and bio-pesticide, had inhibitory effect on germination and emergence of lettuce, mustard (*Sinapis arvensis*), bean, carrot (*Daucus carota*), radish (*Raphanus sativus*), alfalfa (*Medicago sativa*) and rice (*Oryza sativa*) (Ashrafi et al., 2008b; Xuan et al., 2004). Inhibited germination was also observed in bean, tomato and pepper when using crude extracts of black nightshade (*Solanum nigrum)*, lamb-squarters (*Chenopodium album)* and chamomile (*Matricaria chamomilla)* weeds (Kadioglu et al., 2005). Crude extracts from roots and leaves of catmint (*Nepeta meyeri)* inhibited seedling growth of barley (*Hordeum vulgare*) and sunflower (*Helianthus annuus*) by 87 and 67%, respectively (Mutlu and Atici, 2009). Soil amended with ryegrass crude extracts inhibited emergence of Korean lawn grass (*Zoysia japonica)* when used as an organic bio-pesticide (Zuk and Fry, 2006). Also, crude extracts of yuzu of peel (*Citrus junos)* had inhibited germination and reduced seedling growth of agricultural crops in 11 families (Fujihara and Shimizu, 2003). Germination and subsequent seedling growth of eggplant, lettuce, spinach (*Spnacea oleracea*), leek (*Allium porrum*), watermelon and tomato were inhibited by more than 90% (Fujihara and Shimizu, 2003), which was comparable to results in this study.

Chemicals that have been implicated in inhibited germination include terpenoids, flavonoids and phenolic compounds (Marcias et al., 2002). In the Cucurbitaceae Family, most plant species contain allelochemicals (Chen et al., 2005), with certain cultivars in this family having auto-allelopathy with strong inhibited germination (Martin and Blackburn, 2003). In the GLT system, it was demonstrated that the potent chemicals from crude extracts of *Cucumis* species were not released from the plant material through microbial activity, but through leaching by irrigation water (Mashela and Nthangeni, 2002; Mabitsela et al., 2004; Mphosi et al., 2004; Ngobeni et al., 2004). In their review of the chemical structures of the 12 cucurbitacins in the Cucurbitaceae Family, Chen et al. (2005) indicated that cucurbitacin A, which occurs in large quantities in fruits and roots of *C. myriocarpus* is the only cucurbitacin that is water-soluble. Cucurbitacin A comprises two toxic compounds, viz. cucumin ($C_{27}H_{40}O_9$) and leptodermins ($C_{27}H_{38}O_8$), collectively referred to as tetracyclic triterpenoids (Chen et al., 2005). Cucurbitacin A confers auto-allelopathy on *C. africanus* and *C. myriocarpus* seeds, which was removed when exposing seeds to 55˚C for 24 h or to running water for 24 h (Mafeo and Mashela, 2006).

Allelopathic interferences are species-specific, but also have intra-species differences (Prati and Bossdorf, 2004). Allelopathic interference thresholds also vary with plant processes involved and the sensitivity of the recipient species (Inderjit and Duke, 2003). Generally, allelopathic inhibitors interfere with key physiological processes in receptor plants, resulting in reduction of plant growth and development (Ashraf et al., 2008a). Results of this study suggested that processes involved in inhibition of emergence were having similar pathways and therefore the similarities in inhibition is reflected by high levels of R-squared values. Crude extracts of *C. myriocarpus* fruits consistently reduced germination in all plant species when used within the range suitable for transplants in suppression of plant-parasitic nematodes. The observations suggest that cucurbitacin A targets processes which have similar activities, which in this case is germination, since emergence of peas is epigeous instead of being hypogeous as in other dicotyledonous plants (Einhellig, 1986).

In conclusion, all the plant species tested do not have auto-allelopathy; the crude extracts from *C. myriocarpus* fruits have had allelopathic effect on emergence of dicotyledonous seeds. Therefore, the material is not suitable

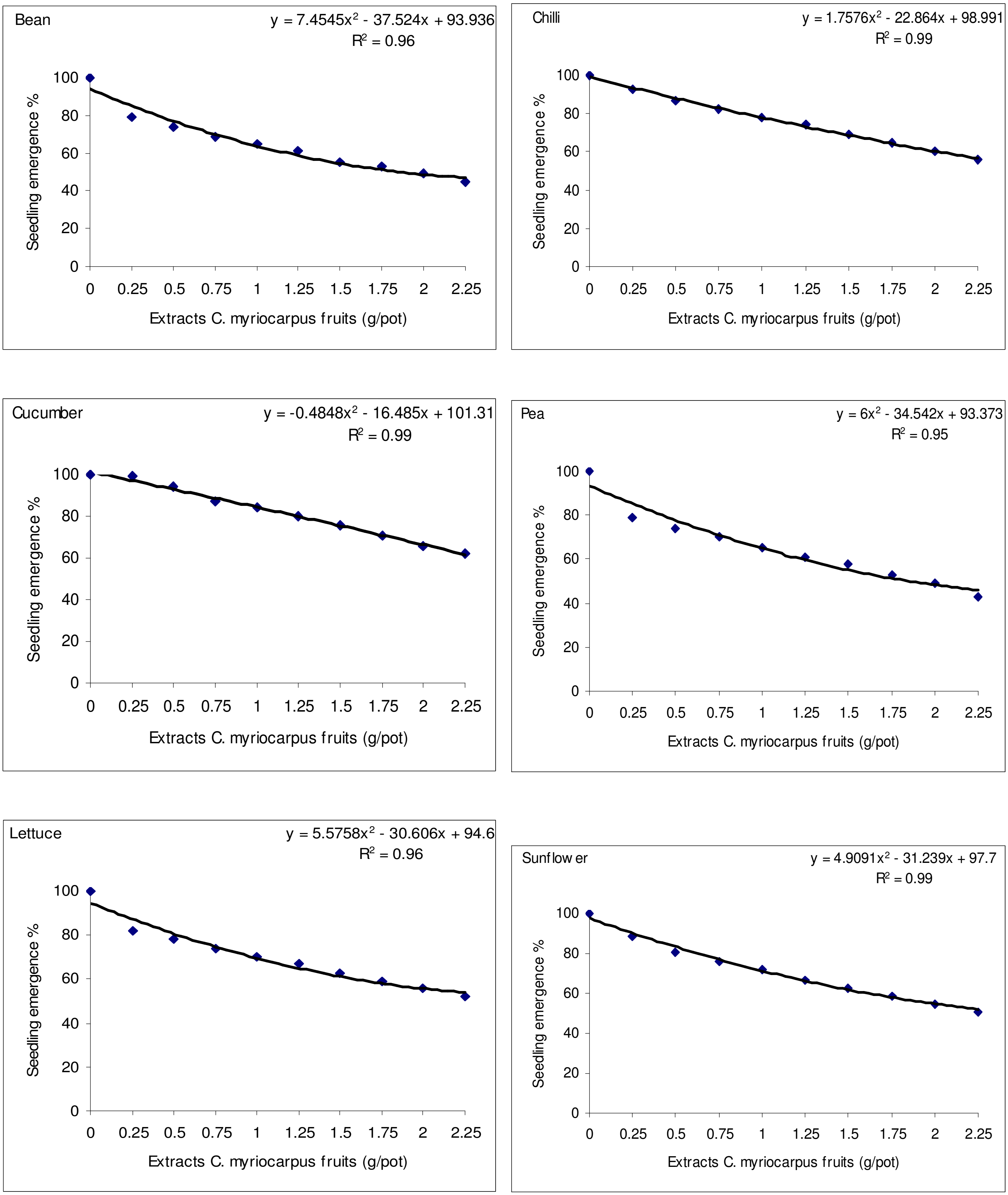

Figure 1. Quadratic relationship between seedling emergence of bean, chili, cucumber, eggplant, lettuce, pea, pepper, sunflower, tomato and watermelon and concentration of crude extracts of *C. myriocarpus* fruits.

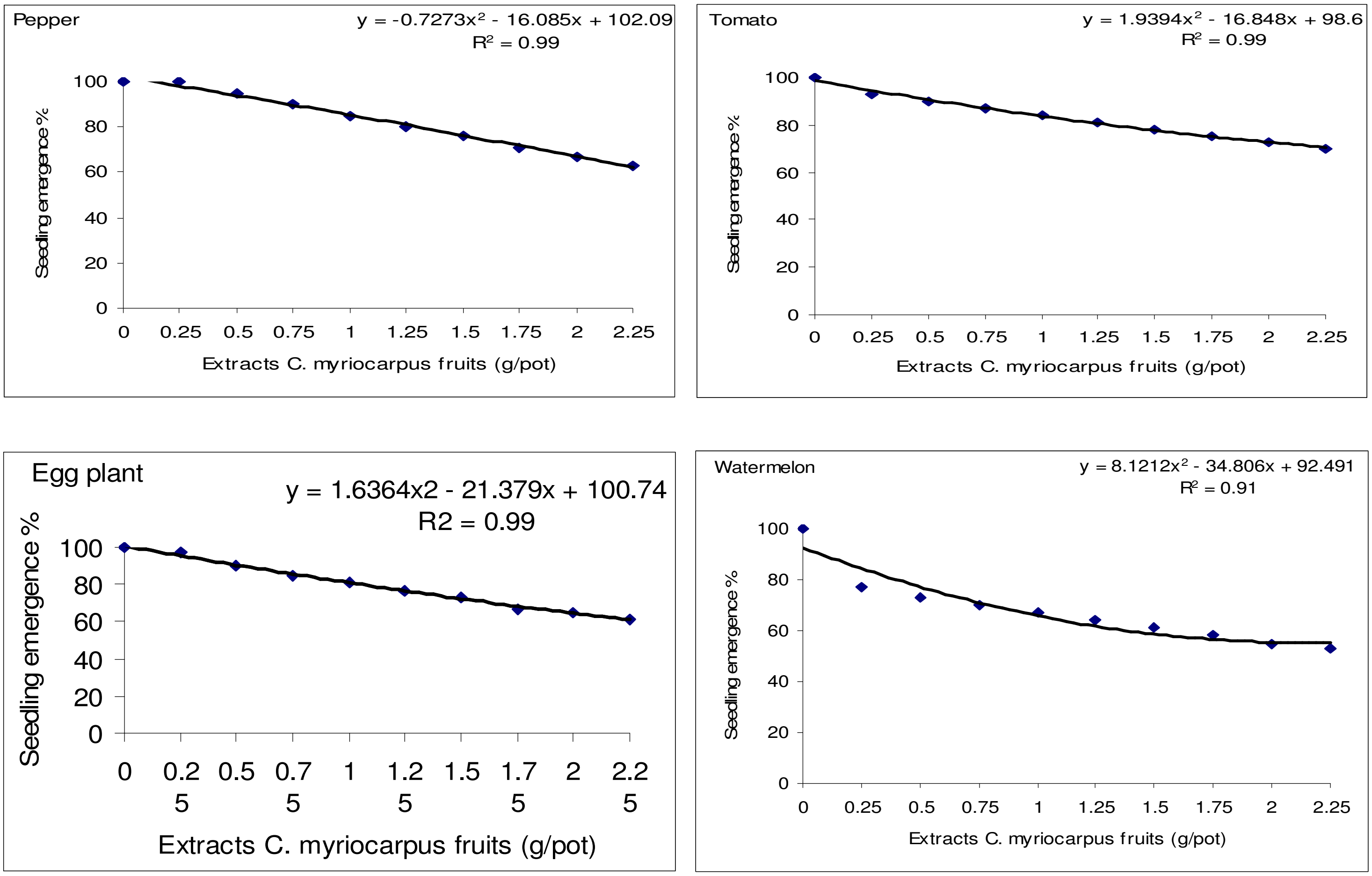

Figure 1. Continued

for use as pre-emergent bio-nematicide.

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