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Full Length Research Paper

Sensitivity of selected Alliaceae seedlings to crude extracts of *Cucumis myriocarpus* fruits

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Crude extracts of wild cucumber (*Cucumis myriocarpus*) fruits have been successfully used as post-emergent bio-nematicide in suppression of the southern root-knot nematode (*Meloidogyne incognita)*. Compatibility of this bio-nematicide with plant growth when used as a pre-emergent bio-nematicide was tested on onion (*Allium cepa)*, leek (*Allium ampeloprasum*) and chive (*Allium schoenoprasum*) under greenhouse conditions. In each trial, 10 levels of crude extracts of *C. myriocarpus* fruits were arranged in a randomised complete block design, with five replicates. At harvest, 18 days after planting, seedling height, radicle length, coleoptile length and coleoptile diameter each were each subjected to the Curve-Fitting Allelochemical Response Data (CARD) model, which demonstrated that at both low and high levels, the bio-nematicide had stimulatory and inhibitory effects on growth of test plants, respectively. The total sum of transformations (∑k), expresses the sensitivity of the test plant to the bio-nematicide, ∑k being inversely proportional to the degree of sensitivity. Overall, onion was the most sensitive to the bio-nematicide, followed by chive and leek. In conclusion, since the material had 100% inhibition of plant growth where it suppresses nematodes, the material is not suitable for use as a pre-emergent bio-nematicide.

Key words: Allelopathy, bio-nematicide, biological systems, CARD model, phytotoxicity.

INTRODUCTION

Plant-parasitic nematodes are among the major constraints in the husbandry of onion (*Allium cepa)*, leek (*A. ampeloprasum*) and chive (*A. schoenoprasum*). Annual crop losses due to plant-parasitic nematodes were estimated at $8 billion in the USA, whereas worldwide estimates are at $78 billion (Koenning et al., 1999; Pang et al., 2009). Specifically, a yield loss in onion due to plant-parasitic nematodes is estimated at 70% (Pang et al., 2009). Yield reduction due to plant-parasitic nematodes is often directly related to pre-plant infestation (Pi) levels (Noling, 1999; Seinhorst, 1965). Large quantities of pre-plant fumigant nematicides had been applied to reduce Pi levels (Dickson and Hewlette, 1988), with environment-unfriendly consequences. Environment-friendly management strategies in plant resistance (Pofu et al., 2010), biological control (Stirling, 1991) and ground leaching technology (Mashela, 2002), are being widely evaluated as alternatives.

The ground leaching technology (GLT) system was developed to ameliorate the drawbacks of using conventional organic amendments in the management of plant-parasitic nematodes, which included: (i) inconsistent nematode suppression results, (ii) large quantities of materials required to suppress nematodes, with the resultant high transport costs and availability, (iii) negative period and (iv) changes in soil pH (Mashela, 2002). The GLT system consistently suppressed nematode numbers, with significant fertiliser effect on crops (Mashela, 2002; Mashela and Nthangeni, 2002; Mashela et al., 2010). The use of crude extracts of wild watermelon (*Cucumis africanus*) and wild cucumber (*C. myriocarpus*) fruits in the GLT system, suggested that the efficacy of the two materials in nematode suppression was similar to that of either aldicarb or fenamiphos (Mashela et al., 2008). The active ingredient involved in nematode suppression from the crude extracts of ground *C. africanus* and *C. myriocarpus* fruits had been identified as cucurbitacin A, which comprises cucumin ($C_{27}H_{40}O_9$) and leptodermin ($C_{27}H_{38}O_8$) (Van Wyk et al., 2002).

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However, a preliminary study suggested that the crude extracts of *C. myriocarpus* fruits used in the GLT system, may not be suitable for use as pre-emergent nematicides to reduce Pi levels in the Solanaceae family due to allelopathic effects on germinating seedlings (Mafeo and Mashela, 2009).

Generally, biological systems respond to extrinsic or intrinsic factors in accordance to the density-dependent pattern, which is characterised by stimulation, saturation and inhibition characteristics (Salisbury and Ross, 1992). Conventional methods of determining density-dependent patterns are tedious (Inderjit, 2001), with inconsistent results. Allelopathy is an extrinsic factor, which should ascribe to density-dependent pattern principles. The Curve-Fitting Allelochemical Response Data (CARD) computerised model was developed to quantify density-dependent allelopathic responses using biological indices (Liu et al., 2003). In the CARD model, the degree of stimulation or inhibition to density-dependent factors like allelochemicals is determined through six parameters, namely: (1) threshold stimulation (D_m) - the dosage at which the allelochemical begins to have a measurable stimulating effect on growth, (2) saturation level (R_h) - the dosage at which growth remains constant prior to decreasing, (3) 0% inhibition (D_0) - the end-point dosage of R_h where the allelochemical has a zero effect on growth, (4) 50% inhibition (D_{50}) - the dosage where the allelochemical inhibits growth by 50%, (5) 100% inhibition (D_{100}) - the dosage where the allelochemical inhibits the growth by 100%, and (6) $R^{2.}$ - the coefficient of determination (Liu et al., 2003).

Quantitative relationships generated by the CARD model are dependent on k, which is the number of ln (D + 1) transformations that serve as a biological indicator of the degree of sensitivity with relation to stimulation or inhibition to allelochemicals (Liu et al., 2003). The lower the ∑k, the higher is the sensitivity of the test plant to the allelochemical and vice versa. The objective of this study was to use the CARD model to determine whether ground extracts of *C. myriocarpus* fruits could serve as pre-plant bio-nematicide in the husbandry of onion, leek and chive.

MATERIALS AND METHODS

Greenhouse studies were conducted at the Horticultural Unit of the University of Limpopo (23°53′10″S, 29°44′15″E), with ambient day/night temperatures averaging 27°C/18°C during autumn. Each crop was assessed in a separate experiment, where fifty 15-cm-diameter plastic pots were placed on the greenhouse bench and filled with 5000 ml growing mixture, comprising 3:1 v/v steam-pasteurised sand and Hygromix (Hygrotech, South Africa). Fruits of *C. myriocarpus* were collected locally, cut into pieces, dried for 5 days at 52°C in an air-forced oven to minimise the loss of volatile phytochemicals (Makkar, 1999) and ground in a Wiley mill through a 1-mm-mesh sieve.

Prior to use, the ground material was stored at room temperature in sealed plastic bags. Onion cv. 'Texas Grano', leek cv. 'Hygrotech G07157' and chive cv. 'Hygrotech J03940 ' were selected as test plants. Ten treatments, viz. 0, 0.25, 0.50, 0.75, 1.00, 1.25, 1.50, 1.75, 2.00 and 2.25 g ground *C. myriocarpus* fruits per pot, were arranged in a complete randomised block design, with five replicates. Pots were irrigated to field capacity prior to planting and then with 250 ml tap water every other day. Two seeds per hole were planted at commercially prescribed depths, with organic amendment being applied in separate holes around the seeds at the same depths and covered with the growing mixture. Plants were thinned to one per pot soon after germination.

Eighteen days after planting, seedling height (cm), radicle length (cm), coleoptile length (cm) and diameter (mm) were measured. Coleoptile diameter was measured using a digital vernier calibre just below the axis. Data were subjected to analysis of variance using the SAS program (SAS Institute Inc. 2004), with means being separated using the Duncan-multiple range test. Significant means ($P \leq 0.01$) were subjected to the CARD model to determine the allelopathic biological indices, namely (D_m), (R_h), (D_0), (D_{50}), (D_{100}), R^2 and k.

RESULTS

In all crops the coefficients of determination were greater than 0.90, suggesting the existence of strong allelopathic interactions. The relationship of the four variables measured and the dosage of ground *C. myriocarpus* fruits were graphically summarised for chive (Figure 1), leek (Figure 2) and onion (Figure 3). Generally, at low dosage the material stimulated growth of all tested organs, whereas at high dosage the material inhibited growth. In chive (Table 1), the transformation levels for seedling height increased from k = 0 (R^2 = 0.89) to k = 6 (R^2 = 0.97). Further increases in k values resulted in the decrease of R^2 to 0.95 at k = 10. Consequently, in chive the best fit to the data for seedling height was at k = 6. Similarly, for the radicle length, coleoptile length and coleoptile diameter in chive, the best fits to the data were at k = 5, k = 5 and k = 8, respectively, in leek at k = 7, k = 20 and k = 15, respectively, and in onion at k = 3, k = 5 and k = 7, respectively (Table 1).

Within the crop, the radicle and coleoptile length of chive had the same k = 5 values, in leek, seedling height and coleoptile length each had a k = 7 value, whereas in onion coleoptile length and diameter had k = 7 values. Among the crops, chive and onion had k = 5 values for radicle length, whereas leek and onion had k = 7 values for coleoptile length. In terms of the model, onion seedling height, with the k = 3 value was the most sensitive to crude extracts of *C. myriocarpus* fruits, whereas leek radicle length with k = 20 was the least sensitive to the material. Overall, onion with ∑k = 22 was the most sensitive to the material, whereas leek with ∑k = 49 was the least sensitive to the material.

DISCUSSION

Generally, at low dosage, extracts of *C. myriocarpus* fruits stimulated growth of various organs in the three test

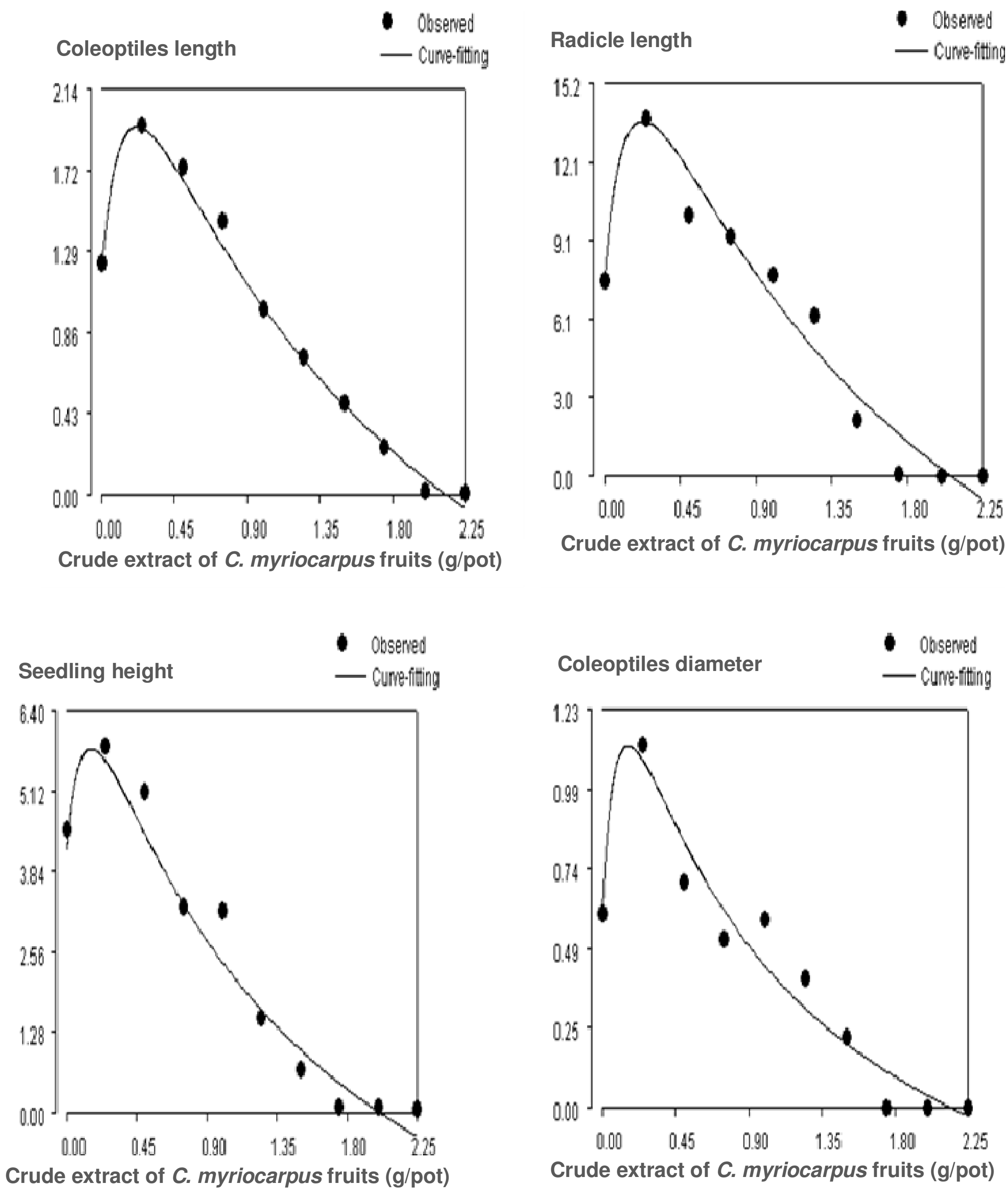

Figure 1. Responses of seedling height, radicle length, coleoptile length and coleoptile diameter of chive seedlings to dosages of crude extracts of *C. myriocarpus* fruits.

plants. In contrast, at high concentrations the material inhibited growth. The stimulation and inhibition responses observed in this study agreed with the major characteristics of density-dependent patterns in various biological systems (Liu et al., 2003). The CARD model results provided an explanation why at low levels, ground *C. myriocarpys* fruits and other materials in the GLT system on tomato plants had a fertiliser effect (Mashela, 2002; Mashela and Nthangeni, 2002; Mashela et al., 2008; Mashela et al., 2010). Generally, in the model, as k increased the R^2 also increased to a peak, where k = i and then decreased from i + 1 transformations until the model ceased running (Liu et al., 2003). The k and R^2 responses in this study agreed with those in Chilli

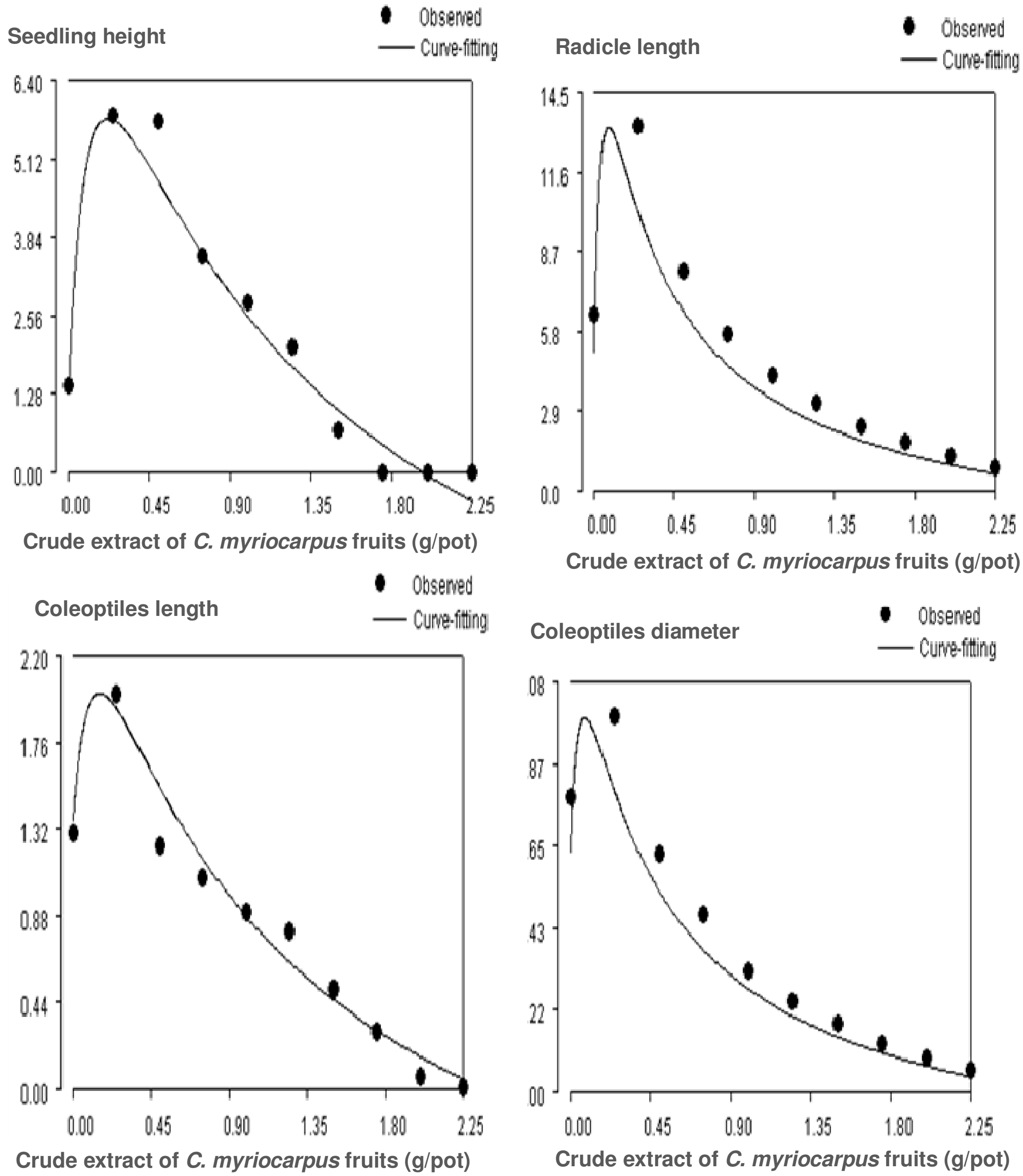

Figure 2. Responses of seedling height, radicle length, coleoptile length and coleoptile diameter of leek seedlings to dosages of crude extracts of *C. myriocarpus* fruits.

(*Capsicum frutescens*), pepper (*C. annum*), eggplant (*Solanum melongena*), and tomato (*Lycopersicon esculentum*) when exposed to crude extracts of *C. myriocarpus* fruits as pre-plant bio-nematicide (Mafeo et al., 2010).

In this study, the ∑k per crop demonstrated that the sensitivity of the measured variables in the three test crops differed with the increasing order of sensitivity to the test material being onion > chive > leek. Similarly, in the Solanaceae family, the sensitivity of the measured variables to crude extracts of *C. myriocarpus* fruits was the highest in eggplant, followed by chili, pepper and then tomato (Mafeo et al., 2010), which explains the successful use of this material in the GLT system on tomatoes (Mashela, 2002; Mashela et al., 2008). The CARD model is useful since it indicates the degree of sensitivity of various organs to the test material, whereas conventional methods are limited to absolute suppression

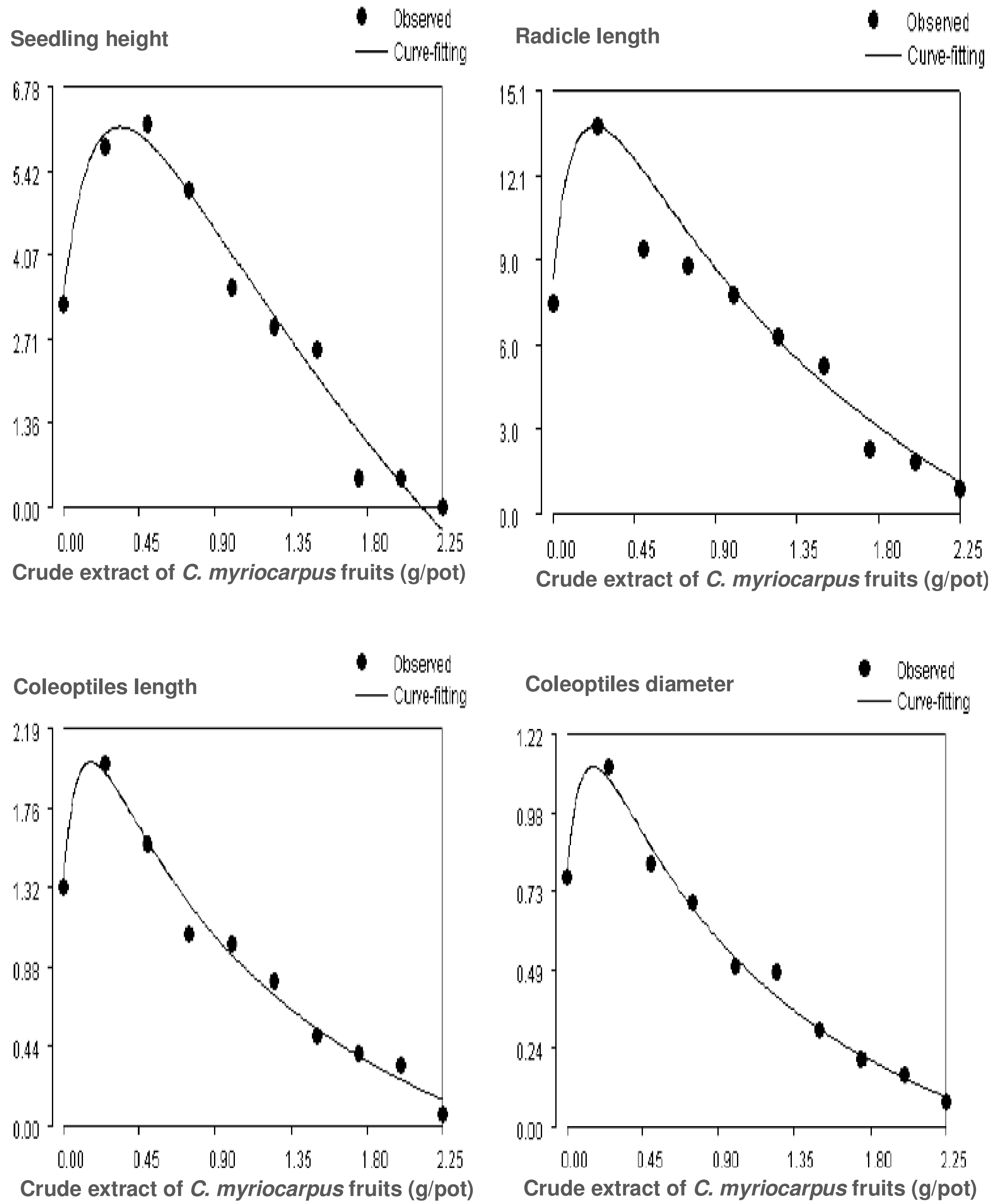

Figure 3. Responses of seedling height, radicle length, coleoptile length and coleoptile diameter of onion seedlings to dosages of crude extracts of *C. myriocarpus* fruits.

of germination (Djurdjevic at al., 2004; Xuan et al., 2004). Also, in these studies and others (Kato-Noguchi, 2003), used concentrations beyond the saturation point, where inhibition sets in. Among the three crops tested using the CARD model, the seedling height in onion was the most sensitive to allelopathic chemicals from the crude extracts *C. myriocarpus* fruits, whereas in leek, the radicle length was the least sensitive.

Generally, 50% inhibition due to crude extracts of *C. myriocarpus* on growth of the test crops occurred at low dosages, whereas 100% inhibition occurred at the dosages where the material was successfully used as a post-planting bio-nematicide (Mashela, 2002; Mashela and Nthangeni, 2002; Mashela et al., 2008). The CARD

Table 1. Responses of four yield components in chive, leek and onion seedlings to dosages from crude extracts of *Cucumis myriocarpus* fruits.

Crops	Model variables	Seedling height	Radicle length	Coleoptile length	Coleoptile diameter
		Dosage of *Cucumis myriocarpus* fruits extracts (g)			
Chive	Threshold stimulation (D_m)	0.16	0.23	0.22	0.15
	Saturation point (R_h)	1.72	5.81	0.78	0.50
	0% inhibition (D_0)	0.56	0.91	0.85	0.75
	50% inhibition (D_{50})	1.09	1.39	1.37	1.25
	100% inhibition (D_{100})	2.00	2.10	2.10	2.10
	R^2	0.97	0.96	0.99	0.94
	k	k = 6	k = 5	k = 5	k = 8
	Sensitivity ranking: ∑k = 24				
Leek	Threshold stimulation (D_m)	0.22	0.08	0.15	0.08
	Saturation point (R_h)	0.77	1.47	0.62	0.43
	0% inhibition (D_0)	1.39	0.68	0.61	0.38
	50% inhibition (D_{50})	1.66	1.23	1.21	0.87
	100% inhibition (D_{100})	2.00	2.90	2.40	2.70
	R^2	0.97	0.99	0.96	0.99
	k	k = 7	k = 20	k = 7	k = 15
	Sensitivity ranking: ∑k = 49				
Onion	Threshold stimulation (D_m)	0.34	0.23	0.16	0.16
	Saturation point (R_h)	0.69	0.97	0.68	0.34
	0% inhibition (D_0)	1.17	0.95	0.67	0.62
	50% inhibition (D_{50})	1.62	1.59	1.32	1.29
	100% inhibition (D_{100})	2.10	2.60	2.60	2.70
	R^2	0.97	0.95	0.98	0.99
	k	k = 3	k = 5	k = 7	k = 7
	Sensitivity ranking: ∑k = 22				

model and the GLT system appear to be much improved tools of quantifying the degree of sensitivity of plant species to allelochemicals when compared with the conventional methods of subjecting test plants to a series of concentrations of extracts from plants with potential allelopathy (Inderjit, 2001).

Conclusion

At the dosage of 2 g/plant where crude extracts of ground *C. myriocarpus* fruits suppressed *M. incognita* race 2, the material had either 50% or 100% inhibition of growth in chive, leek and onion during the 18-day testing period. Consequently, the material is not suitable for use as a pre-plant bio-nematicide in the husbandry of any of the three vegetable crops.

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