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Full Length Research Paper

Modelling responses of maize, millet and sorghum seedlings to crude extracts of *Cucumis myriocarpus* fruit as pre-emergent bio-nematicide

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Biological indices generated using the CARD (curve-fitting allelochemical response data) model may be useful in crop-bio-nematicide interactions since they provide information related to the stimulation dosage of the material to the crops. The objective of this study was to determine: (1) Whether selected crops in the Gramineae family respond to crude extracts of *Cucumis myriocarpus* fruit in density-dependent patterns, and (2) the appropriate dosage of crude extracts of *C. myriocarpus* fruit when used as a pre-plant bio-nematicide in Gramineae family. Separate studies using three maize, millet and sorghum were conducted under greenhouse conditions with ten treatments (0, 0.25, 0.50, 0.75, 1.00, 1.25, 1.50, 1.75, 2.00 and 2.25 g material/pot), arranged in randomised complete block design, with five replicates. At harvest, 18 days after treatment, the CARD model demonstrated that the responses of plant organs when regressed on a series of crude extracts of *C. myriocarpus* fruit stimulate exhibited the density-dependent growth patterns, with various organs having different sensitivities to the material. The integrated sensitivity of the measured variables showed that the test crops differed with increasing order of sensitivity to the test material with increasing order of sensitivity to the material being sorghum > maize > millet. Estimated mean dosage response for stimulation (MDRS) for maize, millet and sorghum being 1.13, 0.86 and 1.12 g, respectively. In conclusion, at low dosages crude extracts of *C. myriocarpus* fruit stimulate plant growth and, therefore, have the potential for use as pre-emergent bio-nematicide in maize, millet and sorghum production.

Key words: Biological indices, CARD model, density-dependent growth patterns, maize, millet, sorghum.

INTRODUCTION

Yield loss in crops due to plant-parasitic nematodes is proportional to the initial population density of nematodes at planting (Seinhorst, 1965). Attempts are being made to develop a pre-emergent bio-nematicide (Mafeo and Mashela, 2010a). However, to date, the promising material had only been successful when used as a post-emergent bio-nematicide (Mashela, 2002). In various studies, germination and emergence of dicotyledonous and monocotyledonous crops and crude extracts of wild cucumber (*Cucumis myriocarpus*) fruit have negative quadratic relationships, which implies that the concentration of crude extracts of *C. myriocarpus* fruit were already beyond the saturation point for germination and seedling emergence (Mafeo and Mashela, 2009a,b; Mafeo and Mashela, 2010a, b). Cucumin, a constituent of cucurbitacin A in crude extracts of *C. myriocarpus* fruit, inhibited division of cancer cells at concentrations where the material was toxic to healthy cells, whereas at lower concentrations, cell division was stimulated (Rimington, 1938). Accordingly, in order to observe the stimulation part of the relationship in plants the dosages had to be drastically reduced.

Quadratic relationships are indicative of biological systems that interact with extrinsic or intrinsic factors in accordance to the density-dependent patterns, which are

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characterised by stimulation, saturation and inhibition growth responses (Salisbury and Ross, 1992). In two separate studies, reproductive factor [RF = (Pf/Pi)] values were regressed on initial population density of nematodes (Pi) (Pofu et al., 2010) and when the concentrations of phenolic compounds were regressed on antioxidants from *Monsonia burkeana* Plant, all exhibited strong density-dependent growth patterns (Mamphiswana et al., 2010). These biological growth patterns provide explanation why literature is replete with inconsistent results for measurement of similar responses under various environmental conditions (Mamphiswana et al., 2010).

The CARD (curve-fitting allelochemical response data) model was developed to quantify responses in biological systems to extrinsic factors in relation to density-dependent growth patterns (Liu et al., 2003). In this model, the degree of sensitivity in stimulation, saturation or inhibition is determined through six biological indices (Mafeo and Mashela, 2010a, b). Relationships generated by the CARD model are dependent on k, which is the number of In (D+1) transformations that serve as a biological indicator of the degree of sensitivity in stimulation, saturation or inhibition (Liu et al., 2003). The lower the ∑k, the higher the sensitivity of the plant to the test material and vice versa. Generally, in the model, as k increases the R^2 also increases to a peak, where k = i and then decreases from i + 1 transformations until the model ceases running (Liu et al., 2003). Biological indices for crops in the Gramineae family in response to the dosage of crude extracts of *C. myriocarpus* fruit are not documented. The objective of this study was to determine: (1) Whether selected crops in the Gramineae family respond to crude extracts of *C. myriocarpus* fruit in density-dependent patterns, and (2) the appropriate dosage of crude extracts of *C. myriocarpus* fruit when used as a pre-plant bio-nematicide in Gramineae family.

MATERIALS AND METHODS

Experiments with three selected crops from the Gramineae family representing economically important crops in Limpopo Province, were conducted under greenhouse conditions at the Horticultural Unit of the University of Limpopo, South Africa (23°53′10″S, 29°44′15″E). Ambient day/night temperatures averaged 27°C/18°C, with maximum temperature controlled using automated thermostat. Each crop constituted a separate experiment, where fifty 15-cm-diameter plastic pots were placed on the greenhouse bench and filled with 5 000 ml growing mixture, comprising 3:1 (v/v) steam-pasteurised sand and Hygromix (Hygrotech, Tzaneen, South Africa). Fruit of *C. myriocarpus* were collected locally, prepared and stored as described previously (Mafeo and Mashela, 2010a).

Maize (*Zea mays* L.) cv. 'SNK 2147', millet (*Panicum miliaceum* L.) cv. 'Babala [OPV]' and sorghum (*Sorghum bicolour* L.) cv. 'Pannar 8609' were selected as test plants.In each experiment, 10 treatments, which are 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* fruit 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 (Hygrotech Planting Chart, 2009), with organic amendment applied in separate holes around the seeds at similar depths and covered with the growing mixture (3 parts sand: 1part Hygromix). Plants were thinned to one per pot soon after germination.

Eighteen days after treatments, seedling height (cm), radicle length (cm), coleoptile length (cm) and coleoptile diameter (mm) were measured. Coleoptile diameters were measured using a digital vernier calibre at 5 cm at the base of sheath of the primary leaves. Data were subjected to analysis of variance using the Statistics 9.0 (Statistics Analytical Software, 1985 to 2008), with treatment means being separated using the Duncan's multiple-range test. Significant treatment means ($P \leq 0.01$) were subjected to the CARD model to determine the biological indices, which are: Threshold stimulation (D_m), saturation level (R_h), 0% inhibition (D_0), 50% inhibition ($D5_0$), 100% inhibition (D_{100}), coefficient of determination (R^2) and number of In (D + 1) transformations that serve as a biological indicator of the degree of sensitivity with relation to stimulation or inhibition to allelo chemicals (k) with integrated sensitivity (∑k) (Liu et al., 2003). Mean dosage response for stimulation (MDRS), which is an estimated dosage for use as pre-emergent bio-nematicide, was computed for maize, millet and sorghum using the relationship: MDRS = $(D_m + R_h)/2$.

RESULTS

Generally, at low dosages, the material stimulated growth of maize (Figure 1), millet (Figure 2) and sorghum (Figure 3), whereas at high dosages the material inhibited growth. In maize (Table 1), the transformation levels for seedling height increased from k = 0 (R^2 = 0.69) to k = 2 (R^2 = 0.84). Further increases in k values resulted in the decrease of R^2 to 0.60 at k = 6. Consequently, in maize the best fit to the data for seedling height was at k = 2. Similarly, for the radicle length, coleoptile length and coleoptile diameter in maize, the best fits to the data were at k = 0, k = 2 and k = 7, respectively. In millet for the three organs the best fit occurred at k = 6, k = 8 and k = 2, respectively (Table 2), whereas in sorghum the best fit was at k = 2, k = 1 and k = 2, respectively (Table 3).

Height and coleoptile length of maize seedlings had the same k = 2 values, while in millet, seedling height and coleoptile diameter each had this value k = 2 value. In sorghum, the radicle length and the coleoptile diameter had the same sensitivities to the crude extracts, that is, k = 2 values. Among the three crops, maize and millet had the same sensitivities (k = 2 values) for seedling height, whereas millet and sorghum had the same sensitivities (k = 2 values) for the coleoptile diameter.

In terms of the model, the radicle length in maize, with k = 0, was the most sensitive to the crude extracts of *C. myriocarpus* fruit, whereas the coleoptile length in millet, with k = 8, was the least sensitive. Overall, sorghum with ∑k = 9 was the most sensitive to the crude extracts, whereas millet with ∑k = 18 was the least sensitive. Recommended mean dosage response values for stimulation (MDRS) using crude extracts of *C. myriocarpus*

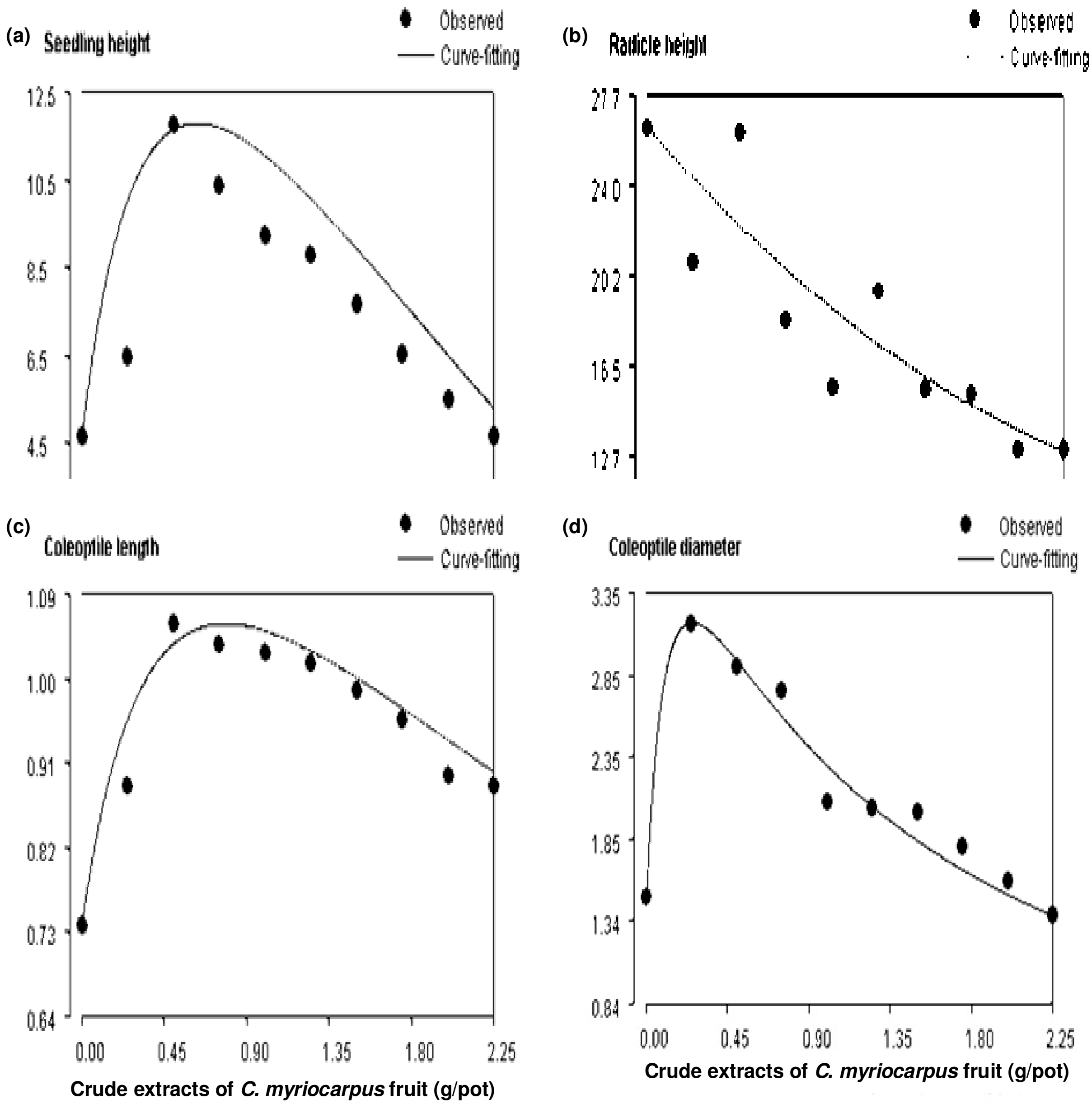

Figure 1. Response of seedling height, radicle length, coleoptile length and coleoptile diameter of maize seedlings to dosages of crude extracts of *Cucumis myriocarpus* fruit at 18 days after planting.

fruit as a pre-emergent bio-nematicide for maize, millet and sorghum were 1.13, 0.86 and 1.12 g, respectively (Table 4).

DISCUSSION

Generally, at low dosages the crude extracts of *C. myriocarpus* fruit stimulated growth of various organs in maize, millet and sorghum, whereas at high dosages the material inhibited growth of the three test crops. Observed stimulation and inhibition growth responses confirmed the major characteristics of density-dependent growth patterns in various biological systems (Liu et al., 2003; Mafeo and Mashela, 2010a; Salisbury and Ross, 1992). Results on the CARD model provided an explanation why at low levels the crude extracts of *C. myriocarpus* fruit and other plant materials in the ground leaching technology system had a fertiliser effect on tomato plants (Mashela, 2002; Mashela and Nthangeni, 2002; Mashela et al., 2008; Mashela et al., 2010).

The integrated sensitivity (∑k) of the measured variables in the three test crops differed with the increasing order of sensitivity to the test material being

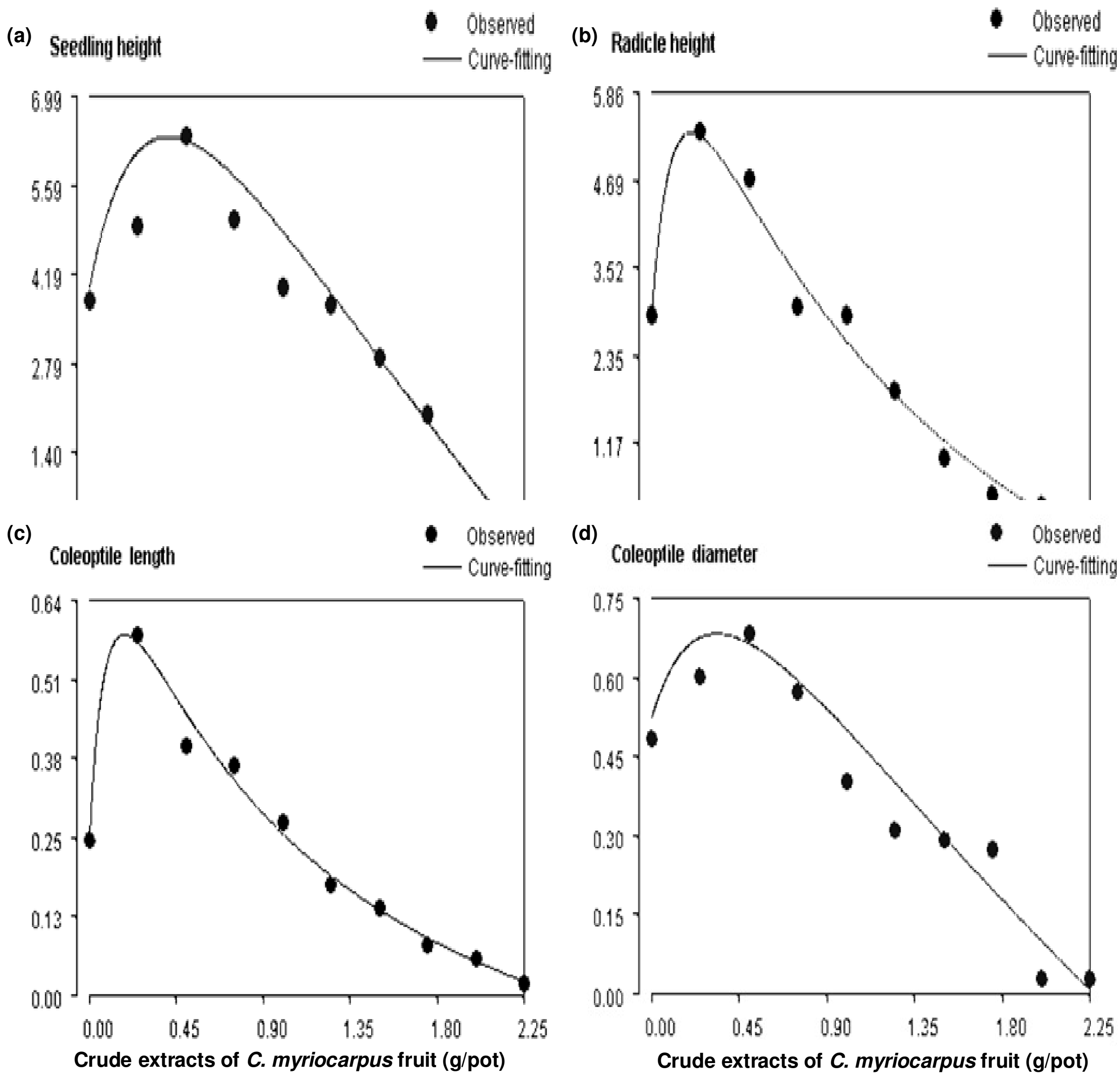

Figure 2. Response of seedling height, radicle length, coleoptile length and coleoptile diameter of millet seedlings to dosages of crude extracts of *Cucumis myriocarpus* fruit at 18 days after planting.

sorghum > maize > millet. Similarly, in the Solanaceae family, integrated sensitivity of the measured variables suggested that eggplant was the most sensitive to the crude extracts, whereas tomato was the leas (Mafeo and Mashela, 2010a). The result explained why this material was successful in the GLT system in tomato production (Mashela, 2002; Mashela et al., 2008). The observation also confirmed the assertion that the degree of sensitivity in plants to allelopathy is plant-specific (Rice, 1984).

The CARD model is useful since it indicated the degree of sensitivity of various organs to the test material, whereas conventional methods are limited to absolute suppression of germination (Djurdjevicet al*.,* 2004; Xuan et al., 2004). Also, in this study and others (Kato-Noguchi, 2003; Mafeo and Mashela, 2009a, b), the model provided concentrations beyond the saturation point, where inhibition occurred. This information is important for future studies where attempts would be made to apply fermented crude extracts of *C. myriocarpus* fruit through irrigation systems. Among the three crops tested, the radicle length in maize was the most sensitive, whereas coleoptile length in millet was the least sensitive. Results of this study demonstrated that the degree of sensitivity to crude extracts of *C. myriocarpus* fruit was also organ-specific.

The active ingredient involved in nematode suppression from the crude extracts of *C. myriocarpus* fruit had been suggested as cucurbitacin A (Mashela, 2002),

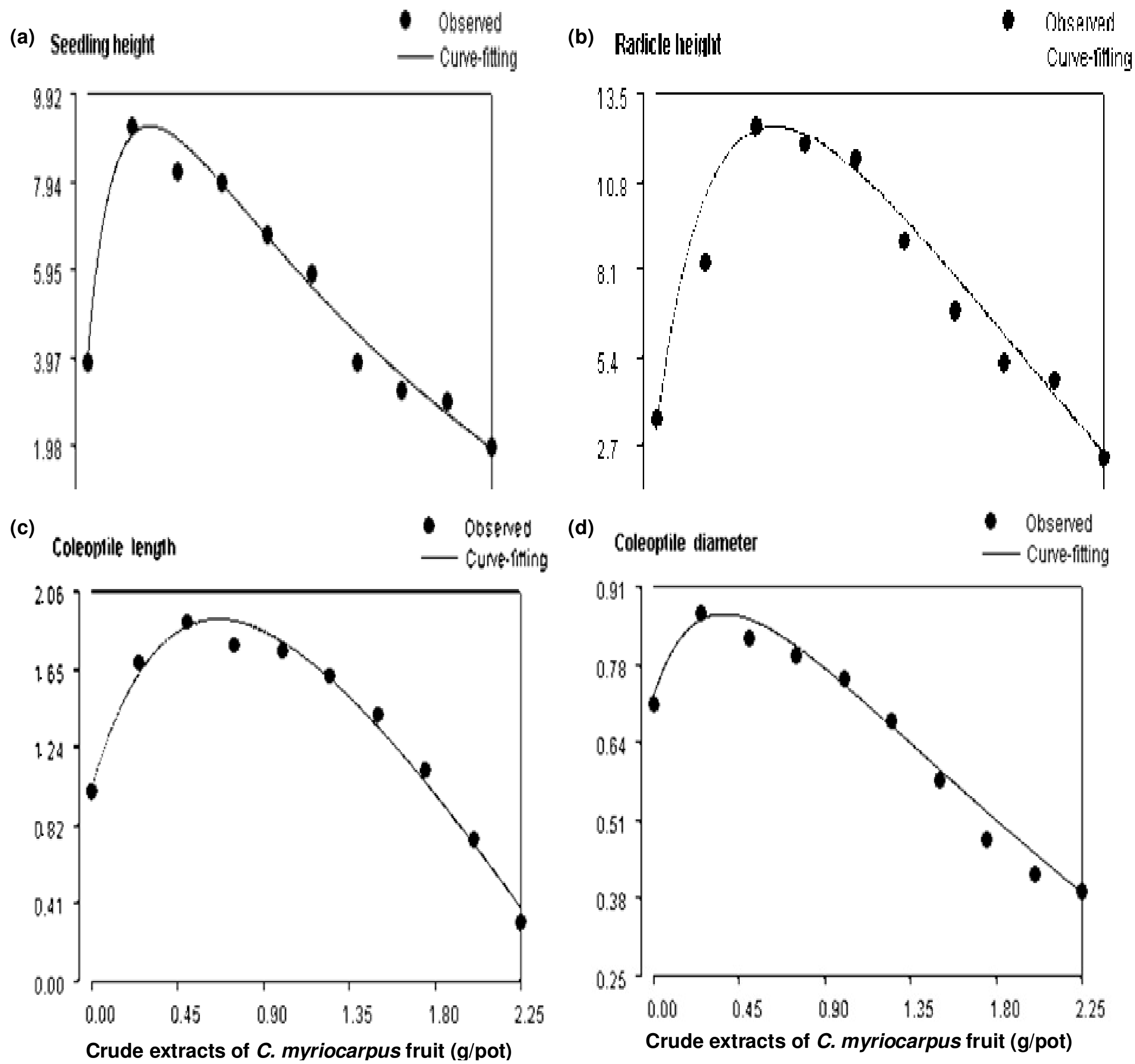

Figure 3. Response of seedling height, radicle length, coleoptile length and coleoptile diameter of sorghum seedlings to dosages of crude extracts of *Cucumis myriocarpus* fruit at 18 days after planting.

Table 1. Responses of four yield components of maize seedlings to dosages from crude extracts of *Cucumis myriocarpus* fruit.

Biological index	Seedling height (cm)	Radicle length (cm)	Coleoptile length (cm)	Coleoptile diameter (mm)	Mean
	Dosage of crude extracts of *C. myriocarpus* fruit (g)				
Threshold stimulation (D_m)	0.63	0.62	0.32	0.26	0.48
Saturation point (R_h)	1.15	3.50	0.79	1.65	1.77
0% inhibition (D_0)	2.39	1.02	3.48	2.15	2.26
50% inhibition (D_{50})	2.89	2.27	7.08	4.65	4.22
100% inhibition (D_{100})	3.40	2.30	12.80	12.8	7.83
Sensitivity K	k = 2	k = 0	k = 2	k = 7	2.75
R^2	0.84	0.78	0.94	0.97	0.88
P- values	≤ 0.01	≤ 0.01	≤ 0.01	≤ 0.01	

Table 2. Responses of four yield components of millet seedlings to dosages from crude extracts of *Cucumis myriocarpus* fruit.

Biological index	Seedling height (cm)	Radicle length (cm)	Coleoptile length (cm)	Coleoptile diameter (mm)	Mean
	Dosage of crude extracts of *C. myriocarpus* fruit (g)				
Threshold stimulation (D_m)	0.41	0.20	0.32	0.15	0.27
Saturation point (R_h)	2.12	2.48	0.81	0.34	1.44
0% inhibition (D_0)	1.23	0.90	1.02	0.94	1.02
50% inhibition (D_{50})	1.72	1.41	1.56	1.59	1.57
100% inhibition (D_{100})	2.20	2.20	2.40	2.30	2.28
Sensitivity (K)	k = 2	k = 6	k = 8	k = 2	4.5
R^2	0.96	0.98	0.99	0.94	0.98
P- values	≤ 0.01	≤ 0.01	≤ 0.01	≤ 0.01	

Table 3. Responses of four yield components of sorghum seedlings to dosages from crude extracts of *Cucumis myriocarpus* fruit.

Biological index	Seedling height (cm)	Radicle length (cm)	Coleoptile length (cm)	Coleoptile diameter (mm)	Mean
	Dosage of crude extracts of *C. myriocarpus* fruit (g)				
Threshold stimulation (D_m)	0.35	0.59	0.67	0.14	0.44
Saturation point (R_h)	5.11	0.88	0.88	0.37	1.81
0% inhibition (D_0)	1.64	2.16	1.78	1.06	1.66
50% inhibition (D_{50})	2.23	2.38	2.16	2.29	2.26
100% inhibition (D_{100})	3.00	2.60	2.50	3.80	2.97
Sensitivity (k)	k = 4	k = 2	k = 1	k = 2	2.25
R^2	0.98	0.96	0.99	0.99	0.98
P- values	≤ 0.01	≤ 0.01	≤ 0.01	≤ 0.01	

Table 4. Mean dosage response for stimulation (MDRS) for using crude extracts of *Cucumis myriocarpus* fruit as a pre-emergent bio-nematicide for maize, millet and sorghum.

MDRS	Crops			
	Maize	Millet	Sorghum	Mean
DRS = (D_m + R_h)/2	1.13	0.86	1.12	1.04

which comprises cucumin ($C_{27}H_{40}O_9$) and leptodermin ($C_{27}H_{38}O_8$) (Van Wyk et al., 2002). In all crops, the dosage response for stimulation quantities intended for use as pre-emergent bio-nematicide were below the amount of 2 g material/plant used at transplanting as post-emergent bio-nematicide in the GLT system. The different quantities take into account the assertion that the degree of sensitivity of plants to allelopathy, in addition to being related to quantity of the material, was also related to the age of the receptor plant (Einhellig and Leather, 1988; Rice, 1984). In all three crops tested, the average dosage response for use as pre-emergent bio-nematicide was 1.04 g material/ plant.

Conclusion

The CARD model demonstrated that the response of maize, millet and sorghum when regressed onto a series of crude extracts of *C. myriocarpus* fruit exhibited the density-dependent growth patterns, which are characterised by stimulation, saturation and inhibition. Using the integration of the responses of various organs to the crude extracts of *C. myriocarpus* fruit within the stimulation range, the quantity of the material which could be used as pre-emergent bio-nematicide in the Gramineae familywas estimated as 1.04 g material/ plant. Evaluating the effect of the proposed MDRS values on initial population densities of plant-parasitic nematodes would give the estimate credibility.

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