

PHYTOTOXIC EFFECTS OF *CITRULLUS COLOCYNTHIS* (L.) SCHRAD ON CERTAIN CROP PLANTS

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Abstract

Effect of *Citrullus colocynthis* on the germination and seedling growth of five crop species was investigated. Aqueous extract of shoot of *Citrullus* affected germination and seedling growth in the order: *Lactuca sativa*, *Sorghum bicolor*, *Brassica rapa*, *Pennisetum americanum*, *Medicago sativa*. Root extract inhibited the germination of only three species in the order: *Pennisetum americanum*, *Sorghum bicolor*, *Brassica rapa* but was stimulatory to *Medicago sativa* and *Lactuca sativa*. Both shoot and root extracts affected root growth to a greater extent than the shoot growth. Decaying shoot and root of *Citrullus* were highly phytotoxic to germination and seedling growth of *S. bicolor*. Artificial rain-drip collected from *Citrullus* leaves arrested seedling growth of *L. sativa* and *S. bicolor*. Coleoptile bioassay of the ether extract indicated some inhibitors in both shoot and root extract and paper chromatography revealed the presence of caffeic and vanillic acids. Possible implications of the results are discussed.

Introduction

The phenomenon of allelopathy, where one higher plant exerts a detrimental influence on another through the production of germination and growth inhibiting substances has been widely reported (Datta & Sinha-Roy, 1975; Naqvi & Muller, 1975; Friedman *et al.*, 1977; Shaukat *et al.*, 1983). The chemical nature of such inhibitors has been reviewed by Bonner (1950), Evenari (1961), and Harborne (1977) and the agricultural and ecological implications of allelopathy are stressed in the reviews by Whittaker & Feeny (1971), Muller & Chou (1972) and Rice (1974). However, comparatively lesser attention has been paid to ascertain the problem of allelopathy among weeds associated with tropical crops.

Citrullus colocynthis (L.) Schrad., a trailing herb of the family Cucurbitaceae occurs both in natural communities on sandy plains, as well as a weed in bullrush millet (*Pennisetum americanum* (L.) Schumann), sorghum (*Sorghum bicolor* (L.) Moench) and other crops in the province of Sind. The density of herbaceous species is generally sparse around *C. colocynthis* in undisturbed vegetation. The influence of *C. colocynthis* on certain crop plants was, therefore, studied to examine the actual mechanism underlying the allelopathic action besides characterizing the phytotoxic principles of this weed.

Material and Methods

1. Phytotoxicity of aqueous extracts of *Citrullus colocynthis* against crop plants: Vigorously growing plants of *C. colocynthis* were collected from *Sorghum* fields near Hub Chowki and dried at 60°C for 3 days. Aqueous extracts were prepared by soaking 10g dry material of fruits, roots and leaves each in 400 ml distilled water for 24 h. Toxicity of the filtrate was tested against *Medicago sativa* L. (local variety), *Lactuca sativa* L. (cv. London Cos), *Brassica rapa* L. (cv. Purple top), *Sorghum bicolor* (L.) Moench (var. White Hegari) and *Pennisetum americanum* (L.) Schumann (var. 1/3 A). Twenty seeds surface sterilized in 2% sodium hypochlorite for 5 min. were placed on Whatman No. 1. filter paper in 9 cm diameter sterile Petri plates, containing 5 ml of the test extract. Deionized distilled water was used as control. Treatments were replicated thrice. Petri plates were kept in a growth chamber (30 ± 2°C) with light intensity of 2000 Lux and photoperiod of 14 h. Germination counts were made daily and the lengths of roots and shoots were recorded after 96 h.

2. Phytotoxicity of decaying *Citrullus colocynthis*: To ascertain the phytotoxic capacity of decaying *C. colocynthis*, dried leaf and root crushed fragments were mixed with sandy loam (76.1% sand, 15.3% silt, 8.6% clay) at the rate of 5, 10 or 20g root or shoot material per 400g soil. These were kept in 8 cm. diameter plastic pots, sprinkled with 100ml water and kept for one week to allow microbial activity. *Sorghum bicolor* (var. White Hegari) was sown, 10 seeds per pot. Each treatment was replicated four times. Germination percentage and root and shoot length of the seedlings was measured after 6 days.

3. Artificial rain and leaching of phytotoxins: The technique used was that of Naqvi & Muller (1975). Small fragments of airdried leaves of *C. colocynthis* placed in a large funnel attached to a conical flask was sprayed with 500 ml of deionized distilled water with a Kiltox sprayer, and the leachate collected. The leachate or the "artificial - rain - drip" was filtered and concentrated to 1/4 in a rotary vacuum evaporator. Phytotoxicity of the leachate was assayed using *S. bicolor* cv. White Hegari and *Lactuca sativa* cv. London Cos.

4. Partial characterization of the phytotoxins:

a) Wheat coleoptile bioassay: Ten g air-dried shoot or root of *C. colocynthis* was blended in 200 ml distilled water. The centrifuged homogenate adjusted to pH 3 with 0.5N H₂SO₄ was extracted three times with peroxidase - free ether and evaporated to dryness over CaCl₂ in a desiccator. To the dry material 2 ml absolute ethanol was added and streaked on Whatman No. 1. filter paper. Duplicate 10 cm wide chromatograms were developed by descending chromatography in iso-propanol: ammonia. water (10:1:1, v/v/v). When the solvent had moved 30 cm from the origin, the chromatograms

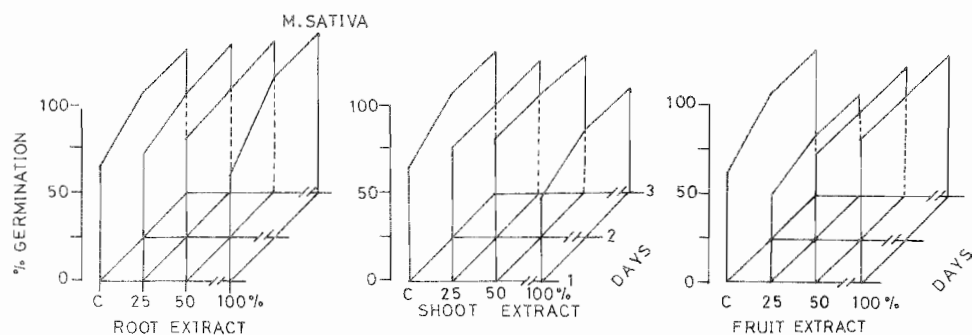


Fig. 1. Effect of root, shoot and fruit extracts of *Citrullus colocynthis* on germination of *Medicago sativa*.

were dried and 10 equal width strips were cut and assayed for growth regulators using wheat coleophile straight growth test of Nitsch & Nitsch (1956).

b) Chromatography: Ether extracts of shoot and root of *C. colocynthis* evaporated to dryness were dissolved in 2 ml ethanol and used for loading Whatman No. 3. filter paper. The chromatograms developed in n-butanol: acetic acid: water (68:10:27, v/v/v) by descending chromatography were examined in UV-light and the phenolic constituents detected using FeCl_3 , $\text{K}_3\text{Fe}(\text{CN})_6$ and vanillin - HCl reagents (Harborne, 1973). Rf-values of the compounds were compared with those reported by Naqvi (1976).

Results

1. Phytotoxicity of *Citrullus colocynthis* against crops:

a) Effect of *C. colocynthis* on germination of crop seeds.

i) *Medicago sativa*: Root extract of *Citrullus* markedly stimulated germination of *Medicago sativa* Fig. 1a) whereas the shoot extract at 100% S reduced the germination ($p < 0.01$). (Fig. 1b). Fruit extract reduced the germination at low concentration but not at high concentration (Fig. 1c).

ii) *Lactuca sativa*: Root extract remarkably increased the rate as well as final percentage germination, at low dosage (Fig. 2a) whereas, the shoot extract at 50% S, delayed germination and at 100% S, inhibited it drastically (Fig. 2b).

iii) *Brassica rapa*: Both root and shoot extracts of *Citrullus* significantly suppressed the rate as well as final percentage germination of *B. rapa* (Fig. 3a, b). The effect was most pronounced at 100% S.

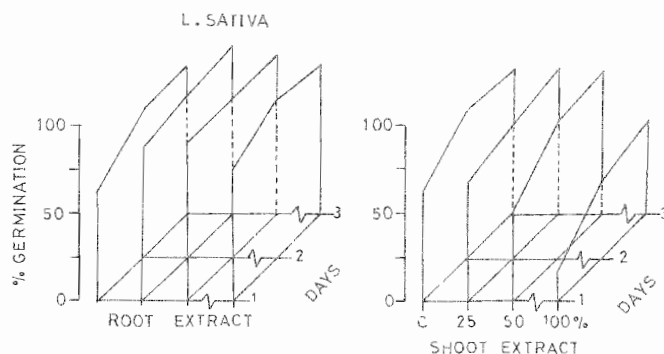


Fig. 2. Effect of root and shoot extracts of *Citrullus* on germination of *L. sativa*.

iv) *Sorghum bicolor*: Rate and the final percentage germination were adversely affected by root as well as shoot extracts of *Citrullus* (Fig. 4a, b). The inhibitory effect enhanced with the increase in concentration. Of the various crop species tested *S. bicolor* seed germination was most affected.

v) *Pennisetum americanum*: Rate as well as final percentage germination of *P. americanum* were substantially reduced by both root and shoot extracts of *C. colocynthis* (Fig. 5a, b). However, the root extract induced greater inhibition of germination than did the shoot extract.

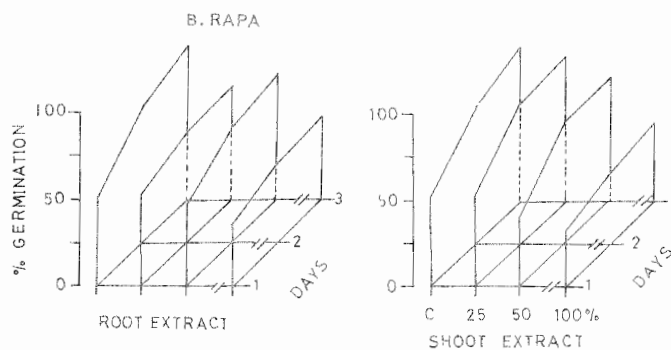


Fig. 3. Effect of root and shoot extracts of *C. colocynthis* on germination of *B. rapa*.

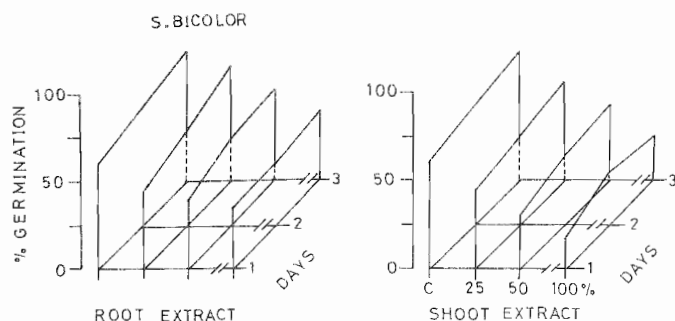


Fig. 4. Phytotoxic effects of *Citrullus* extracts on germination of *S. bicolor*.

b) Effect of *C. colocynthis* on early seedling growth of crop plants:

i) *Medicago sativa*: Shoot extract of *Citrullus* markedly promoted root growth of *M. sativa* at 50% S ($p < 0.01$) but inhibited it at 100% S ($p < 0.001$). Shoot growth remained unaffected by this extract at all the dosages (Fig. 6a). Root extract significantly retarded root growth of *M. sativa*, and inhibitory effect increased with increasing concentration (Fig. 6b), The shoot growth of *M. sativa* remained uninfluenced by root extract. The fruit extract at 25 and 50% S strongly inhibited the root growth (Fig. 6C) but slightly promoted the shoot growth at 100% S.

ii) *Lactuca sativa*: The shoot extract exerted a marked inhibitory effect on the root and shoot growth but only at 100% S (Fig. 7) and root extract only slightly decreased the root growth of at 50 and 100% S ($p < 0.05$) (Fig. 8).

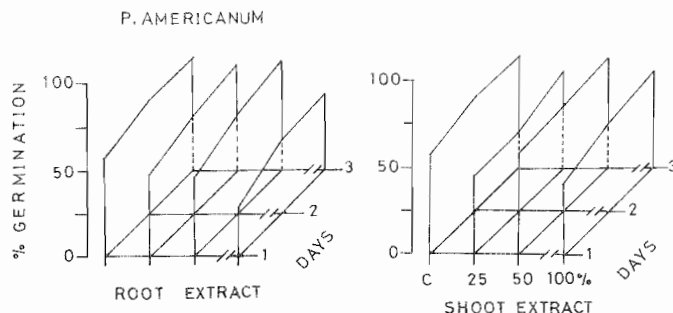


Fig. 5. Effect of *Citrullus* extracts on germination of *P. americanum*.

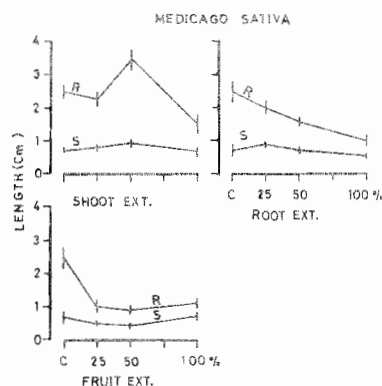


Fig. 6. Effect of root, shoot and fruit extracts of *Citrullus* on early seedling growth of *M. sativa*.

iii) *Brassica rapa*: Shoot extract inhibited the root growth of *B. rapa* at all the concentrations ($p < 0.001$) but substantially promoted the shoot growth ($p < 0.001$) at all dilutions (Fig. 7). A similar response in shoot and root growth was produced by the root extract (Fig. 8).

iv) *Sorghum bicolor*: Shoot extract of *Citrullus* retarded the root growth of *S. bicolor* at all the dosages ($p < 0.01$) but suppressed shoot growth at 50 and 100% S only (Fig. 7). The root extract was comparatively less phytotoxic (Fig. 8).

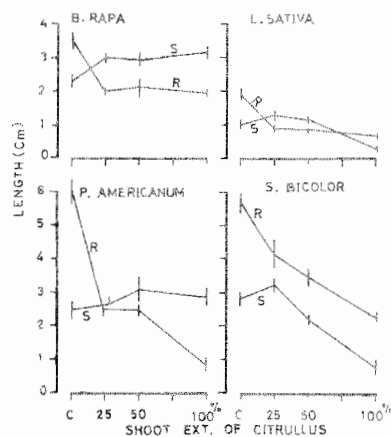


Fig. 7. Phytotoxic effects of *Citrullus* shoot extract on early seedling growth of various test species.

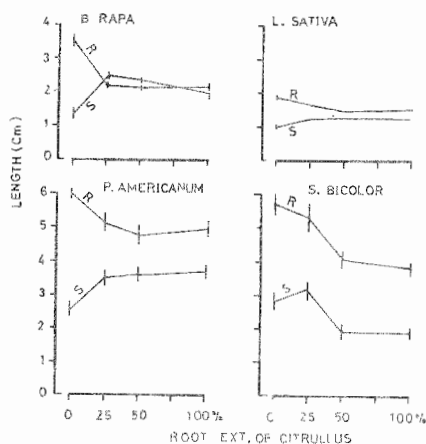


Fig. 8. Phytotoxic effects of *Citrullus* root extract on early seedling growth of various test species.

v) *Pennisetum americanum*: In the presence of shoot extract the root growth was strongly inhibited but shoot growth remained unaffected (Fig. 7). In contrast, the root extract significantly retarded the root growth at 50 and 100% S but shoot growth was stimulated at all the dosages ($p < 0.01$) (Fig. 8).

2. Phytotoxic effects of decaying *C. colocynthis* on germination and growth of *S. bicolor* in soil:

The germination was reduced in soils incorporated with 10 and 20g decaying *Citrullus* shoots ($p < 0.05$) (Table 1). However, decaying roots had no significant effect. Both shoot and root growth were remarkably suppressed in soils containing 10 and 20g *Citrullus* shoot. Decaying root material brought about significant inhibition of shoot and root growth only at 20g (Table 1).

3. Effect of artificial-rain-drip on germination and seedling growth of *L. sativa* and *S. bicolor*:

The original (1x) as well as concentrated (4x) leachates did not significantly alter the germination percentage of either *L. sativa* or *S. bicolor*, however, seedling growth of both the species was invariably affected (Table 2). The leachates did not significantly influence the shoot growth of *L. sativa* but root was retarded at both 1x ($p < 0.05$) and 4x ($p < 0.01$) concentrations. The shoot growth of *S. bicolor* was suppressed only at 4x but the root growth was remarkably inhibited at either dosages ($p < 0.001$) (Table 2).

Table 1. Effect of decaying *Citrullus colocynthis* on germination and seedling growth of *Sorghum bicolor*.

	% Germination	Shoot length cm.	Root length cm
Control	87.00 ± 2.50	13.86 ± 2.03	8.98 ± 1.69
5g Shoot	85.00 ± 2.89 n.s.	14.53 ± 1.72 n.s.	7.59 ± 1.55 n.s.
10g "	77.50 ±* 2.50	9.35 ±*** 1.21	6.25 ±*** 1.11
20g "	75.00 ±* 2.89	8.51 ±*** 0.87	5.77 ±*** 1.25
5g Root	90.00 ± 0.00 n.s.	13.65 ± 1.29 n.s.	9.22 ± 2.11 n.s.
10g "	82.50 ± 2.50 n.s.	12.63 ± 1.71 n.s.	8.76 ⁵ 1.97 n.s.
20g "	82.50 ± 2.50 n.s.	9.02 ±*** 1.03	6.03 ±*** 1.19

*. $p < 0.05$; **, $p < 0.01$.

4.3. Preliminary characterization of the phytotoxins in *C. colocynthis*:

a) Wheat coleoptile bioassay: The bioassay of the ether extract of shoot (Fig. 9) disclosed significant amounts of growth inhibitors at Rf values of 0.5 – 0.6 and 0.8 – 1.0 (referred to as inhibitors A and B respectively in Fig. 9). The assay also revealed the presence of various growth promoters in the shoot extract. The root extract also showed the presence of two inhibitory zones (Fig. 9) at Rf values of 0 – 0.1 and 0.8 – 1.0 (referred to as inhibitor C and B respectively in Fig. 9). Thus the inhibitor B appears to be common to both the extracts.

Table 2. Effect of "artificial-rain-drip" through *Citrullus* leaves on germination and early seedling growth of *Lactuca sativa* and *Sorghum bicolor*

Concentration of leachate	% germination	<i>Lactuca sativa</i>		% germination	<i>Sorghum bicolor</i>	
		Shoot length	Root length		Shoot length	Root length
Control	90 ± 0.0	1.02 ± 0.12	2.23 ± 0.18	78.33 ± 1.66	2.98 ± 0.17	5.27 ± 0.23
1 X	90 ± 2.88 (n.s.)	1.13 ± 0.17 (n.s.)	1.68* ± 0.12	80.00 ± 0.0 (n.s.)	2.79 ± 0.23 (n.s.)	4.01*** ± 0.11
4 X	86.66 ± 1.66 (n.s.)	0.98 ± 0.21 (n.s.)	1.32** ± 0.09	76.66 ± 1.66 (n.s.)	2.33* ± 0.10	3.43*** ± 0.08

*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

b) Chromatographic study: When chromatograms of shoot and root extracts, developed in n-butanol – acetic acid – water were examined in UV-light or sprayed either with ferric–chloride–ferricyanide reagent or with vanilline – HCl reagent they disclosed three spots each. Two of these phenolic compounds were tentatively identified as caffeic and vanillic acid on the basis of matching the experimental Rf value with the standard ones and by their characteristic colour reaction with the reagents. One phenolic compound in each of the sample remained unknown.

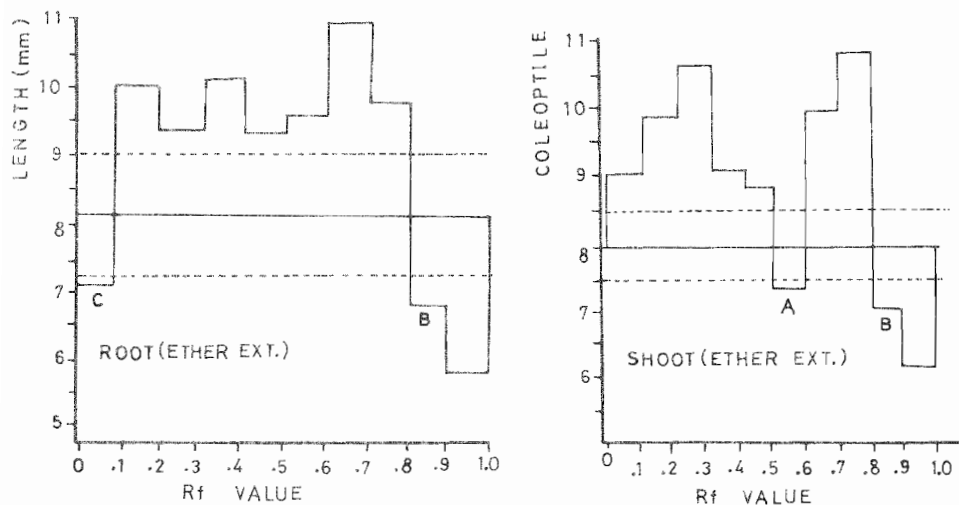


Fig. 9. Histograms of ether fraction of root and shoot extracts of *Citrullus colocynthis* chromatographed on whatman No. 1 filter paper and developed in solvent, isoparapanol: Ammonia: water (10:1:1, v/v/v). Dotted lines represent 95% confidence interval.

Discussion

The results indicate that an aqueous extract of shoot of *C. colocynthis* was more inhibitory to germination as compared to the root extract. Root extract of *Citrullus* increased germination of *Medicago sativa* and *Lactuca sativa* but markedly suppressed germination of *Pennisetum americanum*, *Sorghum bicolor* and *Brassica rapa*. The aqueous extracts of plants are known to inhibit seed germination (Naqvi & Muller, 1975; Friedman *et al.*, 1977, Shaukat *et. al.*, 1983). Plant extracts with promotory effects on germination are also not uncommon (Tripathi & Srivastava, 1970). The inhibitory effect of shoot extract on germination was presumably due to phenolic substances confirmed by chromatographic analysis. Inhibition of germination by phenolic compounds has been reported (Massart, 1957; Evenari, 1961; Naqvi, 1976). The stimulatory effect of root extract at low concentration on germination could be due to growth promoting substances present in the root extract as observed in the coleoptile bioassay.

The shoot extract of *Citrullus* was more phytotoxic to seedling development than was the root extract which even promoted seedling development, particularly the shoot growth in some instances. It retarded root growth of all the species tested, however, arrested shoot growth of *S. bicolor* and *L. sativa* only. The root extract of *Citrullus* suppressed the root growth of all the species tested. Shoot growth was inhibited only in *S. bicolor*. The effect of phytotoxins from *Citrullus* shoot and root appears to be species specific as not all species were equally susceptible to the extract. The species specificity of phytotoxins has also been demonstrated by Naqvi & Muller (1975); Friedman *et al.*, (1977) and Shaukat *et. al.*, (1983). This phenomenon is due to inherent differences in physiological and morphological characteristics of the various species involved.

The decaying shoot of *Citrullus* was more detrimental to germination and seedling growth of *S. bicolor* than the decaying roots. Wilson & Rice (1968) have reported both stimulatory and inhibitory effects on various species with as little amount as 1g of decaying sunflower leaves in 454g soil (2/3 soil + 1/3 sand). Datta & Sinha-Roy (1975) obtained significant reduction in percentage germination with 5 and 10g decaying *Croton bonplandianum* leaves per 250g soil in 13 out of 15 species tested. In such studies the phytotoxins are more effective in coarse textured soils than in fine-textured soils where they get irreversibly adsorbed on colloidal particles. Furthermore, the importance of phytotoxic substances as edaphic variable is greater in deserts where the process of leaching is restricted owing to scanty and erratic rainfall. The presence of toxic residues from various decaying species (that contain phytotoxins) in soil not only determine the composition and dynamic of other species (Parenti & Rice, 1969; Muller & Chou, 1972) but also, in crop fields may seriously affect the establishment and productivity of crops (cf. Rice, 1974). Thus, it is possible that phytotoxins released from *Citrullus* may accumulate in soil in biologically significant amounts and play a key role as a habitat variable, exerting a causative influence on the growth and development of other plants in its vicinity.

Table 3. Rf values (x 100) of phenolic principles in ether fraction of aqueous extracts of *Citrullus colocynthis* and their reactions to various reagents.

Phenolic compound	Rf value		Colour		
	Root	Shoot	UV	Vanillin-HCl	Ferric Chloride-Ferri-Cynide Reagent
Caffeic acid : S	79.0	71.0	Bright blue flor.	Blue	Deep blue
: E	80.0	80.0	Bright blue flor.	Blue	Deep blue
Vanillic acid :S	90.0	90.0	Absorbent	Blue	Sky blue
:E	90.0	90.0	Absorbent	Blue	Sky blue

S = Standard; E = Experimental.

The inhibitory nature of artificial-rain-drip suggests that the phytotoxins present in *C. colocynthis* are highly water soluble and under natural conditions it is very likely that these may be washed out from the leaves by rain, fog or dew into the soil where they would exert inhibitory effect on germination and growth of neighbouring plants. Several reports have appeared regarding the chemical nature of inhibitors produced by higher plants (Whittaker & Feeny, 1971; Rice, 1974; Harbourne, 1977). The coleoptile bioassay of the ether extract of *C. colocynthis* revealed the presence of two inhibitory zones (A and B) in the shoot extract and the same number in the root extract (B and C). Paper chromatography of the extracts tentatively suggested the identity as of the inhibitors caffeic and vanillic acids. However, the presence of other kinds of phytotoxic substances, apart from phenolic compounds, such as terpenoids, alkaloids, glucosides and other secondary metabolites cannot be ruled out as their analysis was not attempted. However, the highly toxic nature of both caffeic and vanillic acid to germination and growth in relatively low quantities has been reported by Naqvi (1976). Phenolic compounds are relatively stable in soil (Minderman, 1968) and consequently they may get accumulated in substantial amounts in soil if prevented from leaching.

Accumulation of toxic principles in biologically significant amounts is likely to affect Bullrush millet and *Sorghum* in fields where *C. colocynthis* grows abundantly and may act as a potent agent in decreasing the crop yields.

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