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AUTOALLELOPATHIC EFFECTS OF FOLIAGE EXTRACTS ON SEED GERMINATION AND SEEDLING GROWTH OF GUAVA (*PSIDIUM GUAJAVA* L.)

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ABSTRACT

The autoallelopathic effects of foliage on seed germination and seedling development of guava (*Psidium guajava* L.) was investigated. Compared with the distilled water control, most of the aqueous extracts of 10, 20, 30, 40, and 50 g dry leaves of guava in a liter of distilled water reduced the final germination percentage, the germination rate, as evaluated by the corrected germination rate index (CGRI), and the radicle growth rate. The detrimental effects on these parameters increased with the increase of the extract concentration. Using mannitol solutions with the same pH and osmotic potentials as the extracts resulted in significantly higher germination percentage and rate and radicle growth rate than most of their corresponding extracts. Consequently, it could be concluded that guava leaves contain allelochemicals which could inhibit seed germination and seedling radical growth of that species.

INTRODUCTION

The guava (*Psidium guajava* L.) is native to tropical America and due to its ease of culture, tolerance to a wide range of climates and altitudes, and high nutritional value it is now distributed to all tropical and subtropical areas of the world (Samson, 1980).

Both sexual and asexual methods are practiced to propagate guava. Stem cuttings, root cuttings, air-layering, and grafting are the

means of vegetative propagation, but they are very difficult and few propagules can be obtained. The propagation by seeds is the most practiced method. Trying to grow guava shoot tips *in vitro*, the first author has noticed that the color of the medium turned brown around the explants which failed to survive (unpublished data). Hence, it would appear that the exudation from the stem tips could be inhibiting to root formation. According to our knwledge there is no information available concerning the nature of this inhibition. The retardation of root elongation in many plant species has been ascribed to water soluble allelochemicals (Yang, 1982 and Warrag, 1995), which usually inhibit seed germination and induce early growth retardation, as well (Rice, 1984; Putnam, 1986; Nurdin & Fulbright, 1990 and Warrag, 1995). This study was conducted to investigate the possibility of allelopathic effects of guava foliage on seed germination and seedling growth. Such studies should help enrich the rather limited research into the allelopathic potentials of crop germplasm (Putnam, 1986) and add to a better understanding of guava.

MATERIALS AND METHODS

Ripe guava fruits were collected from seedy mature trees. The fruits were blended and the seeds were separated by a 1.0 mm wire mesh. The seeds were washed with distilled water, air dried, and stored in paper bags at room temperature.

Fully expanded green leaves were randomly hand-picked from mature guava trees. These trees were used as replicates. The leaves were quickly washed with distilled water, oven-dried at 40° C for 72 h and then stored in polyethylene bags at room temperature. To make the aqueous solutions, 10, 20, 30, 40, and 50 g of dried leaves were soaked in a liter of distilled water and agitated on a shaker at 150 rmp/min for 24 h. The mixtures were then strained through two layers of cheese cloth and filtered through Whatman No. 1 filter papers. The pH and osmotic potentials of the extracts and distilled water control were measured with Metrohm 744 pH meter (Metrohm Ltd., CH-9101 Herisau, Switzerland) and a freezing point depression osmometer (Osmette, Model 5004, Precision System Inc.), respectively. The solutions were kept in a refrigerator at 3° C.

Experiment 1:

Ten ml of the distilled water control and of each of the leaf extracts were dispensed in a 9.5 cm glass Petri dish lined with Whatman No. 1 filter paper. Hundred seeds of guava were uniformly spaced in each Petri dish. The seeds were transferred to new Petri dishes every day to prevent contamination. The Petri dishes were placed in polyethylene bags, to prevent evaporative water loss, and arranged in a completely randomized design inside an oven maintained at 27±2° C. The seeds were examined daily under a dissecting microscope, fitted with a micrometer. Germinated seeds were counted and discarded daily, until no further germination was noticed for six successive days. A seed was considered germinated when the radical protruded by at least≥0.2 mm. Seed germination percentage was determined and the germination rate index (GRI) was calculated as the summation of the daily germination percentage divided by the total number of days of germination (Maguire, 1962). The corrected germination rate index (CGRI) was calculated by dividing GRI by the final germination percentage and then multiplying by 100 (Evetts & Burnside, 1972 and Hsu et al., 1985).

Experiment 2:

Some seeds of guava were surface-sterilized by 10% Clorox (5.25% Sodium hypochlorite) and a few drops of Tween-20, and they were rinsed four times with sterilized distilled water. Five seeds were placed into 150x 250 mm test tubes containing 15 ml of different leaf extract media solidified by 7 g agar l^{-1} after the adjustment of the pH to 5.7. All media were autoclaved at 121° C for 20 minutes. Ten test tubes were assigned per treatment per replicate. All test tubes were maintained at 27±2° C, under a light regime of 2500-3000 Lux, provided by
cool-white fluorescent tubes 16 h daily.
After germination, one seedling was left in each test tube. The hypocotyl and the radicles lengths were determined every other day for 15 days after germination to determine the seedling growth rate.

Experiments 3 and 4:

According to the results of the previous experiments, these experiments were conducted to determine whether the effects of leaf extracts were due to allelochemicals or to the osmotic potential of the

media. Hence, mannitol solutions with the same osmotic potential as the extracts were prepared (Thrill et al., 1979 and Ibanze & Passera, 1997).

$$W\ (g) = P.V.M/R.T$$

where W= weight of mannitol (g), P= desired osmotic potential (bar), V= volume (L), M=molecular weight of mannitol (182.17g), R= gas constant (0.08205), and T= absolute temperature ($T={}^{O}C+ 273$).

The pH of each solution was adjusted to the same level of its corresponding leaf extract. Using the extracts and the mannitol solutions, two experiments similar to 1 and 2 were conducted to determine the seed germination and the lengths of hypocotyl and radicals in the same way as described in experiments 1 and 2. Split plot design was used, with the osmotic potential and pH levels as main treatments and the solutions as subtreatments.

The seed germination percentages were arcsin transformed, then all data were subjected to analysis of variance. Mean separation was performed according to DMRT (Gomez and Gomez, 1984).

RESULTS

The pH and osmotic potential of the extracts were lower than that of the distilled water control (Table 1). Both parameters decreased with the increase of the extract concentration.

All extracts exhibited lower final germination percentage than the distilled water control (Table 2). Progressively less percentage of seeds germinated with the increase of extract concentration. The corrected germination rate index (CGRI) also decreased with the increase of the extract concentration. However, the CGRI level exhibited by the least concentrated extract was as high as that of distilled water (Table 2), whereas the rest of the concentrations were significantly lower. The seed testa in the two least concentrated extracts had the same colour as the distilled water control, while they became increasingly darker in colour with the increase of extract concentration.

Fifteen days after the start of germination, both hypocotyls and radicles were longer in distilled water than in the extracts (Table

2). However, the reduction in length caused by the extract was substantially greater with the radicals than with the hypocotyls. In fact, with the exception of the least concentrated extracts, the radical length in the extracts was almost lacking. In contrast, there was no significant differences in the hypocotyl lengths among the extracts. The colour of the radicles was darker in the most concentrated three extracts.

Compared with the extracts of the same osmotic potentials and pH, mannitol solutions resulted in significantly higher seed germination percentage and CGRI, with the exception of the least and the least two concentrated mannitol solutions for the two parameters, respectively (Table 3). Although, these two parameters decreased with the increase of mannitol solution concentration, the differences were significantly less than those in the extracts. Regarding seedling growth, statistically significant difference in hypocotyl length was brought about by only the most concentrated mannitol solution. On the other hand, radicles were significantly longer in all mannitol solution than in their corresponding extracts. The seed testa and the radicles in the mannitol solutions exhibited the same colour as in the distilled water control, whereas they were darker in colour in the most concentrated three extracts.

DISCUSSION

The results clearly indicated the failure of a significant percentage of the seeds to germinate, especially in the three most concentrated extracts (Table 2). Likewise, the germination rate was considerably reduced, as evaluated by the CGRI, an index widely used to compare the relative rate of germination (Hsu et al., 1985 and Nurdin & Fulbright, 1990). Moreover, the elongation of the radicles of the germinated seeds was substantially retarded by the extracts. Hence, most likely, these seedlings would be as useless as ungerminated seeds.

These effects of the extracts could have been brought about by the osmotic potential, the pH and/or the toxic effects of these extracts (Evenari, 1949; Bell, 1974 and Warrag, 1994, 1995). However, the levels of the osmotic potential (Bell, 1974) and the pH of the extracts were not low enough to be responsible for such

effects. This was clearly indicated by the significantly higher germination percentage, the seed germination rate, as evaluated by the CGRI, and the radicle growth rate, exhibited by the mannitol solutions, in comparison with most of their corresponding extracts (Table 3). Hence, it could be deduced that the guava leaf extracts contained water soluble autotoxins that could detrimentally affect seed germination percentage and rate and seedling radicle growth rate of guava. Apparently, these autotoxins were also responsible for the darker appearance of the seed testa and the radicles, in comparison with the distilled water control. These symptoms have been reported with many other plant species exposed to phytotoxins (Weston & Putnam, 1985; Yang, 1982 and Bialy et al., 1990).

Perhaps, these autotoxins might be responsible for the failure of root formation on stem cuttings. The characterization and identification of these toxins may contribute to their allelopathic mechanism and, hence, to the use of stem cuttings as successful propagules.

REFERENCES

AL-Wasel, A. S. Micropropagation of guava (Psidium guajava L.). Unpublished data.

Bell, D. T. 1974. The influence of osmotic pressure in tests of allelopathy. Trans.III. State Academy of Sci. 67: 312-317.

Bialy, Z., Oleszek, W., Lewis, J., and Fenwick, G. R. 1990. Allelopathic potential of glucosinolates (Mustard oil glycosides) and their degradation products against wheat. Plant and Soil 129: 277-281.

Evenari, M. (1949). Germination inhibitors. Bot. Rev. 15: 153-194.

Evetts, L. L. and Burnside, O. C. (1972). Germination and seedling development of common milk weed and other species. Weed Science 20: 371-378.

Gomez, K. A. and Gomez, A. A. (1984). Statistical procedures for agricultural research (2nd eds.). John Wiley, New York, pp. 680.

Hsu, F. H. Nelson, C. J., and Matches, A. G. (1985). Temperature effects on germination of perennial warm-season forage grasses. *Crop Science 25:215-220.*

Ibanze, A. N. and Passera, C. B. (1997). Factors affecting the germination of albaida (*Anthyllis Cytisoides* L.), a forage legume of the Mediterranean coast. J. Arid Environ. 35: 225-231.

Maguire, J. D. (1962). Speed of germination-aid in selection and evaluation for seedling emergence and vigor. Crop Science 2: 176-177.

Nurdin, and Fulbright, T. E. (1990). Germination of 2 legumes in leachate from introduced grasses. J. of range manag. 43: 466-467.

Putnam, A. R. (1986). Allelopathy: can it be managed to benefit horticulture.HortScience 21: 411-413.

Rice, E. L. (1984). Allelopathy (2nd eds). Academic press, London, pp. 432.

Samson, J. A. (1980). Tropical fruits. Longman Inc., New York, pp. 214-250.

Thrill, D. C., Schirman, R. D., and Appleby, A. P. (1979). Osmotic stability of Mannitol and Polyethylene glycol 20,000 solutions used as seedgermination media. Agron. J. 71: 105-108.

Warrag, M. O. A. (1994). Autotoxicity of mesquite (*Prosopis juliflora*) pericarps on seed germination and seedling growth. J. of Arid Environ. 27: 79-84.

Warrag, M. O. A. (1995). Autotoxic potential of foliage on seed germination and early growth of mesquite (*Prosopis juliflora*). J. of Arid Environ. 31: 415-421.

Weston, L. A. and Putnam, A. R. (1985). Inhibition of growth, nodulation, and nitrogen fixation of legumes by quakgrass. Crop Science 25: 561-565.

Yang, H. J. (1982). Autotoxicity of *Asparagus officinalis* L. J. of Amer. Soc. for Hort. Sci. 107: 860-862.

Table 1: The osmotic potential and the pH of the distilled water control and the different concentrations of guava aqueous leaf extracts.

Germination/growth medium	Osmotic potential (-mPa)	pH
Distilled water	0.000	6.52
Extract concentration (g l^{-1})		
10	0.009*±0.001	5.73±0.02
20	0.030±0.002	5.48±0.04
30	0.055±0.003	5.07±0.02
40	0.082±0.002	4.62±0.03
50	0.101±0.003	4.59±0.04

* Mean of five samples ± standard error of the mean.

Table 2: Response of the final seed germination percentage, the corrected germination rate index (CGRI), the hypocotyl and the radicle length after 15 days of germination of guava to the distilled water control and different concentrations of guava leaf extracts.

Parameter	Distilled water	Leaf extract concentration (g l^{-1})				
		10	20	30	40	50
Germination (%)	84.7* a	77.2b	65.8c	58.0d	40.6e	31.2f
CGRI (day^{-1})	76.5a	73.1a	60.9b	52.4c	37.8d	22.6e
Hypocotyl length (mm)	19.2a	17.4a	16.0ab	15.8b	15.2bc	14.8c
Radicle length (mm)	32.8a	9.5b	2.1c	1.2c	1.1c	1.1c

* Means in the same row followed by different letters were significantly different at the 5% level, by DMRT.

Table 3: The final germination percentage, the corrected germination rate index (CGRI), the hypocotyl and the radicle lengths 15 days after germination of guava seeds under distilled water control and different concentrations of guava leaf aqueous extracts and mannitol solutions.

Germination/growth medium	Osmotic potential (-mPa)				
	0.009	0.030	0.055	0.082	0.101
	Germination %				
Leaf extracts	74.6	60.2	53.9	42.0	34.5
Mannitol solutions	76.5	74.2	73.8	69.1	65.3
Significance of F	**	ns	*	**	**
	CGRI (day $^{-1}$)				
Leaf extracts	72.1	66.2	52.7	40.2	24.8
Mannitol solutions	70.4	65.1	64.8	61.7	57.4
Significance of F	**	ns	ns	*	**
	Hypocotyl length (mm)				
Leaf extracts	21.1	19.8	17.8	17.5	15.3
Mannitol	19.8	19.8	18.3	18.0	17.8
Significance of F	*	ns	ns	ns	ns
	Radicle length (mm)				
Leaf extract	7.3	3.0	2.1	1.5	1.2
Mannitol solutions	29.6	26.4	22.7	23.2	21.8
Significance	**	**	**	**	**

ns= P> 0.05, *= P≤ 0.05, **= P≤ 0.01.

التأثير الذاتي لمستخلص أوراق الجوافة على إنبات بذور ونمو بادرات الجوافة

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ملخص

أجري هذا البحث لدراسة تأثير المستخلص المائي لـ ١٠، ٢٠، ٣٠، ٤٠، و ٥٠ جرام من أوراق الجوافة المجففة لكل لتر من الماء المقطر على إنبات بذور ونمو بادرات الجوافة بالمقارنة مع الماء المقطر (معاملة المقارنة). أدت معظم المستخلصات إلى انخفاض نسبة الإنبات ومعدل الإنبات ومعدل نمو الجذير، وأزداد الانخفاض بازدياد تركيز المستخلص. أدى استخدام محاليل المانيتول المماثلة للمستخلصات في الرقم الهيدروجيني والجهد الأسموزي إلى زيادة معنوية في نسبة الإنبات وكذلك في معدل الإنبات ومعدل نمو الجذير بالمقارنة مع المستخلصات المماثلة للمحاليل. عليه يمكن استنتاج أن أوراق الجوافة تحتوي على بعض المثبطات الكيماوية الذائبة في الماء التي تؤدي إلى منع إنبات البذور وانخفاض معدل الإنبات ومعدل نمو الجذير في الجوافة.