Effect of Potato Alkaloids on the Growth of Alternaria solani and their Possible Role as Resistance Factors in Potatoes

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ABSTRACT

Solanine, chaconine, and solanidine inhibited the radial growth of *Alternaria solani* on potato-dextrose agar. Solanidine was the most inhibitory of the three alkaloids, followed by chaconine and solanine, respectively. At a concentration of 250 ppm, solanine caused a 33% inhibition of growth after a 96-hr incubation period at 24 C. Chaconine was twice as inhibitory, producing a 56% inhibition of growth at a concentration of 250 ppm. Young (30-day-old) potato leaves contained 1,570 ppm of the glycoalkaloids, whereas senescent (120-day-old) leaves contained only 260 ppm. An increased susceptibility of leaves to *A. solani* was correlated with a decrease in glycoalkaloid concentrations in leaves as the potato plants aged. The results suggest a possible role for alkaloids in restricting lesion development in young leaves of the potato plant infected with *A. solani*.

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Early blight caused by *Alternaria solani* (Ell. & G. Martin) Sor. is an important disease of both potatoes and tomatoes. When late blight is absent or is nonepidemic, early blight is the principal defoliation disease on potatoes and tomatoes in the northern United States. Because the most severe attacks usually occur late in the season and on the lower, and thus physiologically older, leaves, early blight is considered a disease of senescent tissues (18, 22, 23). In this connection it has been noted that early-maturing varieties of potato (9) and tomato (22) are generally more susceptible, as are less adequately nourished plants that tend to mature more rapidly (22).

It has been suggested that a temporary resistance, which is governed by physiological factors, exists in young leaves of both tomato and potato. Rowell (18) found that young leaves of tomato and potato can harbor the pathogen without showing visible signs of infection. Only minute lesions are formed. On older leaves on the same plants, the disease develops rapidly and may even kill many of the lowest leaves. Alteration of host physiology by aging, by shading the leaves, or by applying certain chemicals (sodium-2,4-dichlorophenoxyacetate or 2,3-dichloro-1,4-naphthoquinone) increased the amount of disease in greenhouse-grown plants. Conversely, pruning of the growing point of the plant increased the resistance of lower leaves. Rowell proposed that this temporary resistance is controlled by carbohydrate metabolism; specifically, the concentration of nonreducing sugars.

Other workers have suggested that steroid glycoalkaloids in potato and tomato tissues are factors in the resistance of these tissues to fungal pathogens. Tomatine from tomato and solanine from potato are toxic to fungi (1, 3, 5, 10, 12, 13), to insects (8, 21), and to nematodes (2). Solanine is not toxic to most bacterial species (13) and does not appear to be involved in the resistance of potatoes to ring rot (14), even though Paquin & Lachance found that solanine did inhibit the growth of *Corynebacterium sepedonicum* (Spieck. & Kott.) Skapt. & Burkh. in culture (15). The potato glycoalkaloids also did not appear to be responsible for the increased resistance of detached potato leaves to lesion development caused by *A. solani* when the leaves were incubated in continuous light (11).

However, the concentration of the potato glycoalkaloids in the younger (upper) leaves is known to be higher than in the older (lower) leaves, and there is a steady decline in glycoalkaloid concentrations in all leaves as the plant matures (17, 24). The lower concentrations of glycoalkaloids in the older leaves and the increased susceptibility of the older leaves to lesion development suggest that the potato glycoalkaloids may be one of the factors involved in the temporary resistance of young potato leaves to lesion development in the early-blight disease. The toxicities of the potato glycoalkaloids to *A. solani* have not been reported as yet. The purposes of this investigation were to determine the toxicities of solanine, chaconine, and solanidine to the early-blight pathogen and to investigate the possible role of alkaloids in controlling the resistance of young potato leaves to lesion development.

MATERIALS AND METHODS.—*Isolation and purification of potato alkaloids.*—A mixture of 0.5 g of potato alkaloids was isolated from potato sprouts by the methods of Wolf & Duggar (24). The mixture of ammonia-precipitable alkaloids was twice recrystalized from ethanol and then dissolved in methanol:water (4:1). Chaconine, solanine, and solanidine were then separated on a basic alumina

column (4). The identities of the alkaloids eluting from the column were determined by chromatography on thin-layer (TL) plates of Silica Gel G (SG). We also made chromatographic comparisons of solanine and solanidine obtained from the potato sprouts with commercial solanine and solanidine (K & K Laboratories, Inc.).

In the TL solvent system for separating tomato alkaloids (4), solanine, chaconine, and solanidine had R_F values of 0.37, 0.52, and 0.85, respectively. Chaconine from potato sprouts had R_F values of 0.52, 0.50, and 0.22 on TL SG plates developed with 95% ethanol: acetic acid: 95% ethanol (1:3), and *n*-butanol: acetic acid: water (10:3:1), respectively. These R_F values were almost identical to those reported in the literature for chaconine in the three solvent systems (3, 16, 25), and solanine was clearly separated from chaconine in all three systems. Commercial solanine contained a trace of chaconine. However, solanine and chaconine isolated from the alkaloid mixture by column chromatography were chromatographically pure. The relationships and chemistry of the potato alkaloids have been summarized by Schreiber (20).

We determined the quantity of each alkaloid in the mixtures isolated from sprouts and from leaves by separating the alkaloids on TL plates in the solvent system described by Arneson (4) and eluting the individual alkaloids from the SG, which had been scraped off in bands. The $SbCl_3$ - HCl reagent developed by Bretzloff (7) was used to determine the quantity of each alkaloid. Commercial solanine served as the standard upon which the determinations were based. Chaconine, solanine, and solanidine all have the same extinction coefficient (16).

Toxicity measurements.—Alkaloids in different concentrations were incorporated before autoclaving into three basal media: Difco dehydrated potato-dextrose agar (PDA); Difco dehydrated lima bean agar (LBA); and Difco dehydrated potato-dextrose broth (PDB). Three ml of the medium were poured into 35 × 10 mm plastic petri dishes. The plates were inoculated with 5-mm agar plugs cut from sporulating cultures of *A. solani*, and the inoculated plates were incubated in the dark at 24 C. Three plates of each treatment were used to estimate the variance; colony diameters were measured to the nearest mm.

Two isolates of *A. solani* were used for the studies: one isolate was from a field-infected leaf of Katahdin growing near Pocomoke, Md.; the other isolate came from an infected tuber of the potato variety White Rose grown in Idaho.

Resistance measurements.—Leaves were removed from the third, fourth, and fifth nodes (from the apex) of greenhouse-grown plants (cultivar Katahdin) at different stages of maturity. Five g of these leaves were used for each alkaloid analysis, and each analysis was replicated 3 times. For the quantitative determinations of leaf alkaloids, the leaves were extracted with ethanol and acetic acid in a Soxhlet apparatus, and the alkaloids precipitated with ammonia as previously described (19).

Leaves from these same nodes were also detached, placed in petri plates with moistened filter paper, and inoculated with 5-μliter droplets of a spore suspension of *A. solani* (ca. 20 spores/μliter). We inoculated the leaves by puncturing them through the droplet with a needle. The inoculated leaves were placed in an incubator at 22 C in the dark. Ten leaves were used for the resistance test at each growth stage. Lesion diameters were measured to the nearest mm after 3 days' incubation.

RESULTS AND DISCUSSION.—*Growth inhibition produced by potato alkaloids.*—At concentrations of solanine as low as 250 ppm, the radial growth of *A. solani* on PDA was inhibited by more than 25% even after 150 hr of growth. When the PDA contained 2,000 ppm solanine, growth was completely inhibited for more than 40 hr. Five hundred ppm solanine caused about 50% growth inhibition (Fig. 1). Both isolates used in this study had the same sensitivity to solanine and to the alkaloid mixture in our tests. McKee (13) found that the growth of *Fusarium caeruleum* (Lib.) Sacc. was not inhibited by the addition of 500 ppm solanine to the PDA medium, whereas the growth of *Helminthosporium carbonum* Ullstrup on PDA was inhibited 50% by 61 ppm (7×10^{-5} molal) solanine (3). Apparently, *A. solani* is more sensitive to the inhibitory action of solanine than *F. caeruleum* but less sensitive than *H. carbonum* to solanine.

H. carbonum is not pathogenic to potatoes, and perhaps the greater sensitivity to solanine of *H. carbonum* in comparison with either *F. caeruleum* or *A. solani* is analogous to the situation with tomato

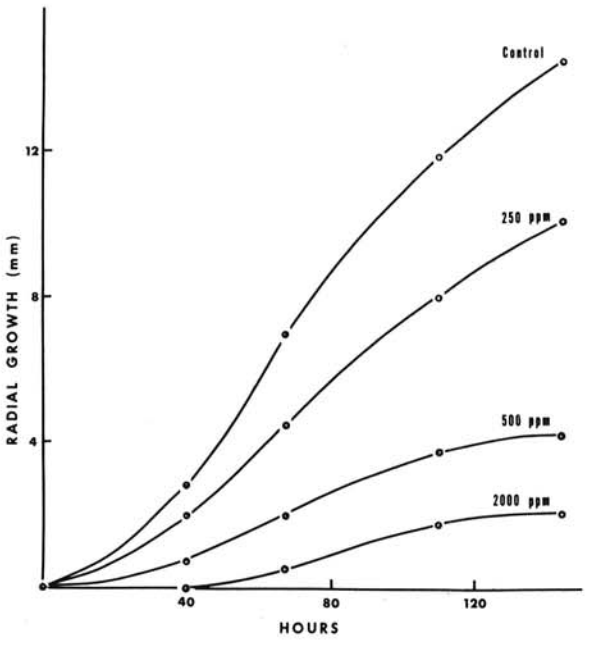

Fig. 1. Effect of solanine on the growth of *Alternaria solani* on potato-dextrose agar.

TABLE 1. The effects of solanine, chaconine, solanidine, and a mixture of the potato alkaloids on the growth of *Alternaria solani* on potato-dextrose agar (PDA) and lima bean agar (LBA)[a]

Alkaloid	Concentration (ppm)	% Inhibition[b]
PDA		
Solanine[c]	250	33
Solanine[d]	250	33
Chaconine[d]	250	56
Solanidine[c]	100	72
Alkaloid mixture[e]	250	40
LBA		
Solanine[c]	250	26
Solanine[c]	500	42
Solanidine[c]	100	67
Alkaloid mixture[e]	250	27
LSD ($P = .05$)		4.8

[a] Radial growth at 24 C.
[b] Inhibition measured 96 hr after inoculation.
[c] Commercial (K & K Laboratories).
[d] Purified from alkaloid mixture on ALO_2 column (see MATERIALS AND METHODS).
[e] Purified from potato sprouts. About two-thirds chaconine, one-third solanine, plus a trace of solanidine.

pathogens and nonpathogens, and the greater sensitivity of nonpathogens of tomato to tomatine (6). Arneson & Durbin (6) found that fungal pathogens of tomato are less sensitive to tomatine than are related fungal species that are nonpathogens or saprophytes.

Because the alkaloids occur as mixtures in potato tissues (14, 15), they were tested individually on the growth of *A. solani*. Solanidine at a concentration of 100 ppm (0.25 molal) was more inhibitory than solanine or chaconine at a concentration of 250 ppm (0.29 molal), and even more inhibitory than solanine at a concentration of 500 ppm (0.58 molal). Chaconine at a concentration of 250 ppm (0.29 molal) was more inhibitory to the growth of *A. solani* than solanine at a concentration of 500 ppm (0.29 molal) (Table 1). The mixture of potato alkaloids, which contained about two-thirds chaconine, two-thirds solanine, and a trace of solanidine, was more toxic than pure solanine but less toxic than pure chaconine (Table 1).

Since solanine and chaconine differ from each other in only one sugar moiety, apparently the differences in toxicity to *A. solani* of these two glycoalkaloids is due to the single sugar substitution. Chaconine is composed of one solanidine aglycone molecule, one D-glucose molecule, and two L-rhamnose molecules, whereas solanine has the same structure except for the substitution of one D-galactose molecule in place of one of the two L-rhamnose molecules found in chaconine.

In agreement with our results, McKee (13) found that chaconine was about 2 times as toxic as solanine to spores of *F. caeruleum*. However, in contrast to our findings, he found that solanidine was much less toxic to spores of *F. caeruleum* than solanine. He attributed the lower toxicity of solanidine to its poor solubility. Arneson & Durbin (4, 5) found that tomatidine, the aglycone of tomatine, was also less toxic than the glycoalkaloid, and they postulated that the enzymatic hydrolysis of the sugar molecules from the intact glycoalkaloid molecule by tomato pathogens was a detoxification mechanism.

The toxicities of both solanine and tomatine to other fungi are greater at higher pH levels (3, 5, 13). Chaconine is also more toxic to nematodes at higher pH levels (2). We found that the inhibition of the growth of *A. solani* by solanine or the mixture of alkaloids was greater at higher pH levels (Table 2). At a concentration of 100 ppm of either solanine alone or the natural mixture of alkaloids, 84% inhibition of growth was produced on PDA buffered at pH 7.6. In contrast, no inhibition from solanine at a concentration of 100 ppm was observed on agar buffered (0.1 M potassium phosphate) at either pH 5.6 or 6.0 (Table 2).

Our results concerning the effects of pH on the toxicities of potato alkaloids confirm the findings of previous studies concerning pH effects with other organisms (2, 5, 12, 13). The undissociated base of these alkaloid molecules is probably the toxic form of the molecule. McKee (13) showed that the portion of solanine present as undissociated base at any particular hydrogen-ion concentration, calculated from the dissociation constant of solanidine, was in fact responsible for most of the toxicity determined experimentally, in the case of the inhibition of spore germination of *F. caeruleum*.

Solanine, solanidine, and the natural mixture of alkaloids also inhibited the growth of *A. solani* on LBA (Table 1). The inhibition on LBA produced by the alkaloids was slightly less than the inhibition on PDA. This is not surprising, since there are many factors that may affect the toxicities of the alkaloids, including such factors as pH, salts in the medium, and sugar concentration of the medium (13). The

TABLE 2. Effect of potato glycoalkaloids and pH of buffered potato-dextrose agar (PDA) on growth of *Alternaria solani*[a]

pH	% Inhibition of growth	
	Solanine (100 ppm)	Alkaloid mixture[b] (100 ppm)
7.6	84	84
7.2	54	66
6.8	14	33
6.4	8	19
6.0	0	15
5.6	0	0
5.6 (unbuffered)	13	18

[a] Buffered with 0.10 M phosphate buffers. Radial growth measured 96 hr after inoculation.
[b] Alkaloid mixture isolated from potato sprouts (two-thirds chaconine, one-third solanine, plus a trace of solanidine).

addition of 2% glucose or 1% $CaCl_2$ to a potato extract medium negated the inhibitory action of solanine on *F. caeruleum* growth (13).

In potato-dextrose broth, the potato alkaloids were also inhibitory to the growth of *A. solani*. At a concentration of 250 ppm, the naturally occurring mixture of potato alkaloids produced a 48% inhibition of the dry wt increase of the fungus, after 8 days' growth at about 22 C. At a concentration of 1,000 ppm, growth was completely inhibited.

After 8 days' growth of *A. solani* in the PDB medium, the pH of the medium had increased from an initial pH of 5.7 to a pH of 7.5. In the medium to which 200 ppm of the mixture of alkaloids was added, the pH also increased from an initial pH of 5.7 to a final pH of 6.8. The pH of the PDA medium, measured with an electrode after 5 days' growth of the fungus, was 5.9 in the buffered medium (0.10 M phosphate at an initial pH of 5.6) and 7.6 in the unbuffered medium (initial pH of 5.6). We obtained these measurements by scraping the mycelium from the surface of the agar and melting the agar. Because the pH of the growth medium affects the inhibition produced by the potato alkaloids (Table 2), it might be expected that the alkaloids would be less inhibitory in a medium buffered at pH 5.6 than in unbuffered PDA where the pH changes to 7.6 after 5 days' growth of the fungus. Indeed, both solanine and solanidine are less inhibitory in PDA buffered at pH 5.6 than in unbuffered PDA, which also has an initial pH of 5.6.

Leaf alkaloid contents and lesion development.—Young leaves from Katahdin plants were more resistant to lesion development in the detached leaf test, and also had higher glycoalkaloid contents than did older leaves (Table 3). Detached leaves from the middle portion of young Katahdin plants (30 days after emergence) developed minute necrotic lesions after 3 days' incubation in the dark at 22 C. The variation between reactions on individual leaves ranged from 0 mm to 2 mm in lesion size. Small lesions (up to 2 mm) also developed on some of the check (H_2O-inoculated) leaves, produced by puncturing the leaves with a needle (Table 3).

No visible lesions were produced after 7 days on the upper and middle leaves of whole plants inoculated 30 days after planting with a spore suspension of *A. solani* and held in a mist chamber for 24 hr. The lowermost leaves developed a few large lesions, and most of these leaves abscised. At later stages of growth (70, 90, 120 days), much larger lesions were produced on detached leaves and on the upper and middle leaves of whole plants (Table 3). These results confirmed the findings of Rowell (18), that young leaves of potato plants are highly resistant or immune to lesion development caused by *A. solani*.

Resistant leaves from 30-day-old plants have higher glycoalkaloid contents than the susceptible leaves from 90-day-old plants (Table 3). The glycoalkaloid concentration of leaves taken from the third, fourth, and fifth nodes decreased with increasing age. In other experiments, leaf glycoalkaloid concentrations as high as 2,200 ppm were found in leaves of just-emerging plants (two- to three-node stage). Although many environmental factors may affect the levels of glycoalkaloids in leaves, there is a steady decrease in the glycoalkaloid levels with increasing age of the plants (Table 3).

TABLE 3. Effect of age of plants on glycoalkaloid content and resistance to lesion development in detached potato leaves[a]

Age of plants (days from emergence)	Glycoalkaloid concentration (ppm, fresh wt)	Lesion[b] size (mm)
30	1,570	0.1 a
70	1,020	2.3 b
90	540	3.1 c
120	260	3.0 c

[a] Inoculated leaves incubated 3 days in dark at 22 C.

[b] Average lesion size: expressed as difference between lesions produced on inoculated leaves and lesions produced on check (H_2O droplets) leaves. Means not followed by the same letter are significantly ($P = .05$) different from each other, using Duncan's multiple range test.

Since concentrations above 250 ppm of solanine or the alkaloid mixture in cultural media can inhibit the growth of *A. solani*, concentrations of the alkaloids above these levels in potato leaves might similarly inhibit the growth of *A. solani* in the leaves. Wolf & Duggar (24) found that glycoalkaloid concentrations in leaves (cultivar Earlaine) ranged from 1,680 ppm (dry wt basis) for leaves from the seventh and eighth nodes, down to 1,000 ppm (dry wt basis) for leaves from the lowest two nodes, in plants at the 15-node stage of development. The highest concentration of glycoalkaloids reported by Wolf & Duggar (24), 9,000 ppm (dry wt basis), in shoot tips of 21-day-old Russet Burbank plants would be about 1,000 ppm on a fresh weight basis, according to their measurements of solids contents. The lowest concentration, 800 ppm (dry wt basis), in leaves from 89-day-old Russet Burbank plants would be about 90 ppm on a fresh weight basis.

From the results we obtained concerning the inhibition of growth of *A. solani* by glycoalkaloids added to the cultural medium, the higher concentrations of glycoalkaloids (>500 ppm, fresh wt basis) found in younger potato leaves could inhibit the growth of the pathogen in vivo, and thus restrict lesion development on younger leaves. The lower glycoalkaloid concentrations (<100 ppm, fresh wt basis) would probably not be inhibitory, as shown by our growth inhibition studies on cultural media, and thus these lower glycoalkaloid concentrations would not inhibit lesion development.

The proposed inhibition of lesion development in the young leaves by the high glycoalkaloid contents of these leaves could be an important factor in the temporary resistance of young potato plants to lesion development caused by *A. solani*. The relative proportion of solanine and chaconine, which varies with age and physiological condition of the leaves

according to Paseshnichenko (17), could be another important factor influencing the resistance of leaves to lesion development, since chaconine was shown to be about 2 times as inhibitory to *A. solani* as solanine. The pH of diseased leaf tissue could also be very important, since hydrogen-ion concentrations greatly affect the inhibitory action of potato alkaloids on the growth of *A. solani* in vitro.

LITERATURE CITED

1. AGERBURG, L. S., R. SCHICK, M. SCHMIDT, & R. V. SENGBUSCH. 1933. Die Bestimmung des Solaningehaltes von Pflanzen mit Hilfe von Cladosporium fulvum. Zuchter 5:272-280.
2. ALLEN, E. H., & J. FELDMESSER. 1971. Nematicidal activity of chaconine: Effect of hydrogen-ion concentration. J. Nematol. 3:58-61.
3. ALLEN, E. H., & J. KUĆ. 1968. α-Solanine and α-chaconine as fungitoxic compounds in extracts of Irish potato tubers. Phytopathology 58:776-781.
4. ARNESON, P. A., & R. D. DURBIN. 1967. Hydrolysis of tomatine by Septoria lycopersici: a detoxification mechanism. Phytopathology 57:1358-1360.
5. ARNESON, P. A., & R. D. DURBIN. 1968. Studies on the mode of action of tomatine as a fungitoxic agent. Plant Physiol. 43:683-686.
6. ARNESON, P. A., & R. D. DURBIN. 1968. The sensitivity of fungi to α-tomatine. Phytopathology 58:536-537.
7. BRETZLOFF, C. W. 1971. A method for the rapid estimation of glycoalkaloids in potato tubers. Amer. Potato J. 48:158-162.
8. DAHLMAN, D. L., & E. T. HIBBS. 1967. Response of Empoasca fabae to tomatine, solanine, leptine I, tomatidine, solanidine, and demissidine. Ann. Entomol. Soc. Amer. 60:732-740.
9. DOUGLAS, D. R., & J. J. PAVEK. 1972. Screening potatoes for field resistance to early blight. Amer. Potato J. 49:1-6.
10. FONTAINE, T. D., G. W. IRVING, JR., R. M. MA, J. B. POOLE, & S. P. DOOLITTLE. 1948. Isolation and partial characterization of crystalline tomatine, an antibiotic agent from the tomato plant. Arch. Biochem. 18:467-475.
11. GOTH, R. W., S. L. SINDEN, & MURIEL J. O'BRIEN. 1969. Effect of light and glycoalkaloids on lesion development caused by Alternaria solani on potatoes. Phytopathology 59:1556 (Abstr.).
12. MC KEE, R. K. 1955. Host-parasite relationships in the dry-rot disease of potatoes. Ann. Appl. Biol. 43:147-148.
13. MC KEE, R. K. 1959. Factors affecting the toxicity of solanine and related alkaloids to Fusarium caeruleum. J. Gen. Microbiol. 20:686-696.
14. PAQUIN, R. 1966. Study on the role of the glycoalkaloids in the resistance of potato to bacterial ring rot. Amer. Potato J. 43:349-354.
15. PAQUIN, R., & R. A. LACHANCE. 1963. Effects des glycoalkaloides de la pomma de terre sur la croissance de Corynebacterium sepedonicum (Spieck. & Kott.) Skapt. & Burkh. Can. J. Microbiol. 10:115-122.
16. PAQUIN, R., & M. LEPAGE. 1963. Separation de la solanine, de la chaconine et de la solanidine per chromatographie sur couches minces. J. Chromatogr. 12:57-62.
17. PASESHNICHENKO, V. A. 1957. Content of solanine and chaconine in the potato during the vegetation period. Biochemistry (USSR) 22:929-931.
18. ROWELL, J. B. 1953. Leaf blight of tomato and potato plants: factors affecting the degree of injury incited by Alternaria dauci f. solani. Rhode Island Agr. Exp. Sta. Bull. 320. 29 p.
19. SANFORD, L. L., & S. L. SINDEN. 1972. Inheritance of potato glycoalkaloids. Amer. Potato J. 49:209-217.
20. SCHREIBER, K. 1968. Steroid alkaloids: The Solanum group, p. 1-192. *In* R. H. F. Manske [ed.]. The alkaloids-chemistry and physiology. Academic Press, New York. Vol. 10.
21. STRUCKOW, B., & I. LOW. 1961. The effects of some Solanum alkaloid glycosides on the potato beetle. Entomol. Exp. Appl. 4:133-142. Chem. Abstr. 56:5156.
22. THOMAS, H. R. 1948. Effect of nitrogen, phosphorus, and potassium on susceptibility of tomatoes to Alternaria solani. J. Agr. Res. 76:289-306.
23. WALKER, J. C. 1952. Diseases of vegetable crops, p. 353-356. McGraw-Hill Book Co., New York. 529 p.
24. WOLF, M. J., & B. M. DUGGAR. 1946. Estimation and physiological role of solanine in the potato. J. Agr. Res. 73:1-32.
25. ZITNAK, A. 1968. Separation of glycoalkaloids from Solanum tuberosum L. by thin-layer chromatography. Can. Soc. Hort. Sci. Proc. 7:75-87.