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Characterization of *Lycopersicon* Species Leaves Anti Fungal Compounds Through Improved Thin Layer Chromatographic Techniques

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Abstract: Experiments were conducted to characterize the *Lycopersicon hirsutum f.typicum* three accession LA.1777, LA.1927, LA.1363 and *L. esculentum* leaf surfaces trichomes exudates and direct leaf disks trichomes, separated on TLC plates in hex/ether (80:20) and tested the anti fungal properties of trichomes against the challenging fungi *C.cucumerinum* Ell. and Arthur. It was found that reduced level to 5ul crude extract of *L.hirsutum* LA. 1777 separated on silica gel plate in hex/ether (80:20) showed significantly strong anti fungal activity and inhibitory zone in the third region. While no fungi toxic activity was detected on TLC for accession, LA.1927, LA.1363 and *L. esculentum* 5ul volume level crude extracts.

Key words: *Lycopersicon* sp., trichome, sesquiterpenes, *Cladosporium cucumerinum,* inoculum, chromatography

INTRODUCTION

Lycopersicon hirsutum Humb. and Bonpl. is a wild relative of the cultivated tomato and very resistant to insect pests (Gentile and Stoner, 1968; Gentile *et al*., 1968; Kennedy and Dimock, 1983). This resistance is believed to be associated with the presence of peculiar foliar trichomes on both the adaxial and abaxial surface of leaves. Glandular trichomes densities and the presence of volatile compounds in trichome gland secretions have been implicated as factors mediating resistance in *Lycopersicon* species (Dimock and Kennedy, 1983; Snyder and Carter, 1985; Weston *et al.,* 1989; Ave and Tingey, 1986). *L.hirsutum* accessions generally display abundant densities of types I, IV and VI trichome type. These compounds are apparently synthesized and secreted from glandular trichomes on the leaf surfaces and characterized the major components as C_{15} compounds, most likely sesuiterpenoids (Harren *et al*., 1987). Most plants do not act as host to the vast majority of potential pathogens, viruses, bacteria, mycoplasm, fungi, nematodes or insects (Esquerre'-Tugaye, 1987). Most of the monoterpeniod compounds showed very strong action against a number of fungi (Thoppil *et al*., 1998). Antibacterial and anti fungal activity of the essential oils obtained from other species of *Lamiaceae* including various *Mentha* species and *Ocium* species etc have been reported where the major constituents in these oils were mostly monoterpenes. Most of the monoterpenoid compounds showed very strong activity against bacteria and also against a number of fungi (Khan and Saeed, 2002). Patterson *et al.* (1975) characterized as sesuiterpenoids several compounds from PI 251303 foliage found to be repellent and/or toxic to *T. urticae* and the hexane extracts of whole *L.hirsutum* leaves or cotton swabs of leaf surfaces were concentrated and separated in non-polar and polar fraction by chromatography. It was found that reduced oviposition by *H. zea* was shown to be related to the frequency of glandular hairs on foliage. Since washing leaves with 75% ethanol reduced resistance (Tigchelaar, 1986). Many plants are used as insecticides molluscides and rodenticide (Poswal *et al.,* 1993, Anwar *et al.,* 1992). The use of plants or plant materials as fungicide is of great importance and need more attention (Bodde, 1982). A bioauto graphic technique and procedure for detecting fungi toxic compounds on thin layer chromatography was followed as developed by Homans and Fuchs; 1970, but with some modifications. In the experiment leaf crude extract and direct leaf disk compounds were separated on TLC in hex/ether (80:20) solvent system before inoculum spray. The objective of this study was to investigate the reaction of leaves trichome exudates and leaf disk of *Lycopersicon hirsutum* three accession and cultivated tomato *L. esculentum* against *cladosporium cucumerinum in vitro.*

MATERIALS AND METHODS

Plant material: *L.. hirsutum f. typicum* wild tomato specie three accessions LA. 1777, LA. 1927, LA. 1363 and one cultivated tomato *L. esculentum* ace plants growing in the green house of Horticulture and landscape architecture

Department, University of Kentucky were selected for the experiment. Hexane crude extract, water extract and direct leaf sample were extensively investigated to find the reaction of active anti fungal compounds against the challenging fungi *cladosporium cucumerinum in vitro*. Using the silica gel TLC (20x20 cm) plate and run the blank plate in hex/ether (80:20) solvent system to wash out any impurities and then dried. TLC plate was then marked with lead pencil in the bottom line at eight different places equally spaced for four accession with two replicates for each treatment Fig. 1. Applied crude extract 5ul sample from accession LA. 1777, LA. 1363, LA. 1927 and *L. esculentum* prepared previously to the marked positions allotted on TLC for the four accession. The sample was air dried for 3-5 minutes. Measured 160 ml hexane and 40 ml ether and mixed together and dispensed in the chamber. The spotted TLC plate was placed in the solvent system for separation of the component parts found in crude extract/and or to divide the various compounds in polar/non-polar fraction compounds. When the solvent reached to the top line the plate was then removed from the chamber and air dried for half an hour. The TLC plate was then observed under UV light and identified the position of fraction compounds from bottom to top as polar and non-polar. The major compounds four distinctive regions were clearly visible under UV light on the experimental plate and the third region area was more significant for *L. hirsutum* three accessions. To verify the trichome exudate reaction phenomenon crude extract of LA 1777 and its leaf disks with no treatment were compared on second and third TLC plate Fig. 2. TLC full size plate was cut into two parts. TLC 1 and TLC 2 and marked the plates bottom line at three positions horizontally. Applied 20ul crude extract to the first left position and then cut one disk 1 cm size from accession LA. 1777 leaflet and put on the second position and lightly pressed with a piece of glass and then removed glass and disk quickly. Again cut two disks from LA. 1777 leaflets and put on the third position one by one and lightly pressed with glass, both the glass and disk were removed. TLC II was marked at two places on the bottom line and cut three leaf disks 1 cm size and pressed lightly on the left first marked position one by one and then removed and thrown away. Applied 20ul LA. 1777 crude extract to the right side marked position. TLC I and TLC II were placed in 200 ml hex/ether (80:20) solvent chamber. When the solvent reached to the top line of plates the chamber was opened and plates removed and then air dried for half an hour. The plats were sprayed with C. *C.cucumerinum* fungal spores inoculum as mentioned below.

***C.cucumerinum* culture and bioassay-preparation:** *Cladosporium cucumerinum* Ell. and Arthur was obtained from plant pathology Department, University of Kentucky and grown on V-8 juice agar plates. Three to four culture plates were sufficient for two chromatographic plates. Fungal spores were obtained from *C.cucumerinum* by flooding two-week old cultures with 50 ml buffer salt solution. Poured the inoculum in chromatographic sprayer and using chromatographic sprayer the entire spore suspension was sprayed as soon as possible on the sample chromatographic plate. The TLC plate was sprayed from left right and up down and then the plate was put in a baking dish Pyrex trays humidity chamber, containing 50 ml Luke warm water and sealed the baking dish with tape and kept at room temperature approximately 22 °C. Similar inoculum spray and bioassay procedure was adopted for the second experiment. The bioassay normally become ready with in 24-48 h.

RESULTS AND DISCUSSION

The three accession LA. 1777, LA. 1927, LA. 1363 belong to *L.hir. f.typicum* group and one *L. esculentum*. ace. The baking dish chamber bioassay plate was opened and thoroughly inspected for fungal growth and inhibitory zones Fig. 1. It was observed that accession LA. 1777, one fraction in both R1 and R2 showed activity which was called later III region. No other constituent parts below or above this region produced any inhibition spots. The other three accessions LA. 1363 and LA. 1927 and *L. esculentum* showed no activity in any region. It was clear that as little as 5ul crude extract application and further separation in a solvent system, the strong anti fungal activity was present only in accession LA. 1777 third region compounds. It was also found that crude leaf extracts of *L. hirsutum* accession LA. 1777 and that of leaf disks one, two and three in number also showed similar regions and inhibitory zones as observed in Fig. 2. (a and b). In preliminary studies conducted in our laboratory (Khan and Snyder 1991, unpublished data) in equal weight leaf samples extracted in hexane and water also showed similar constituents third region compounds and same anti fungal properties, but not shown here. Significant amounts of sesquiterpenes and α. santalen and zingeberene have been found in extracts of two wild tomato species (Coates *et al*., 1988). Juvik *et al.* (1988) had a microscopic viewing of leaflets of LA.1777 and PI. 365936 revealed a dense vestiture of what appear to be type IV trichomes, each with a tiny droplet of exudate on their tips. The abundance of these trichomes and the apparent amount of total exudate suggest they are the

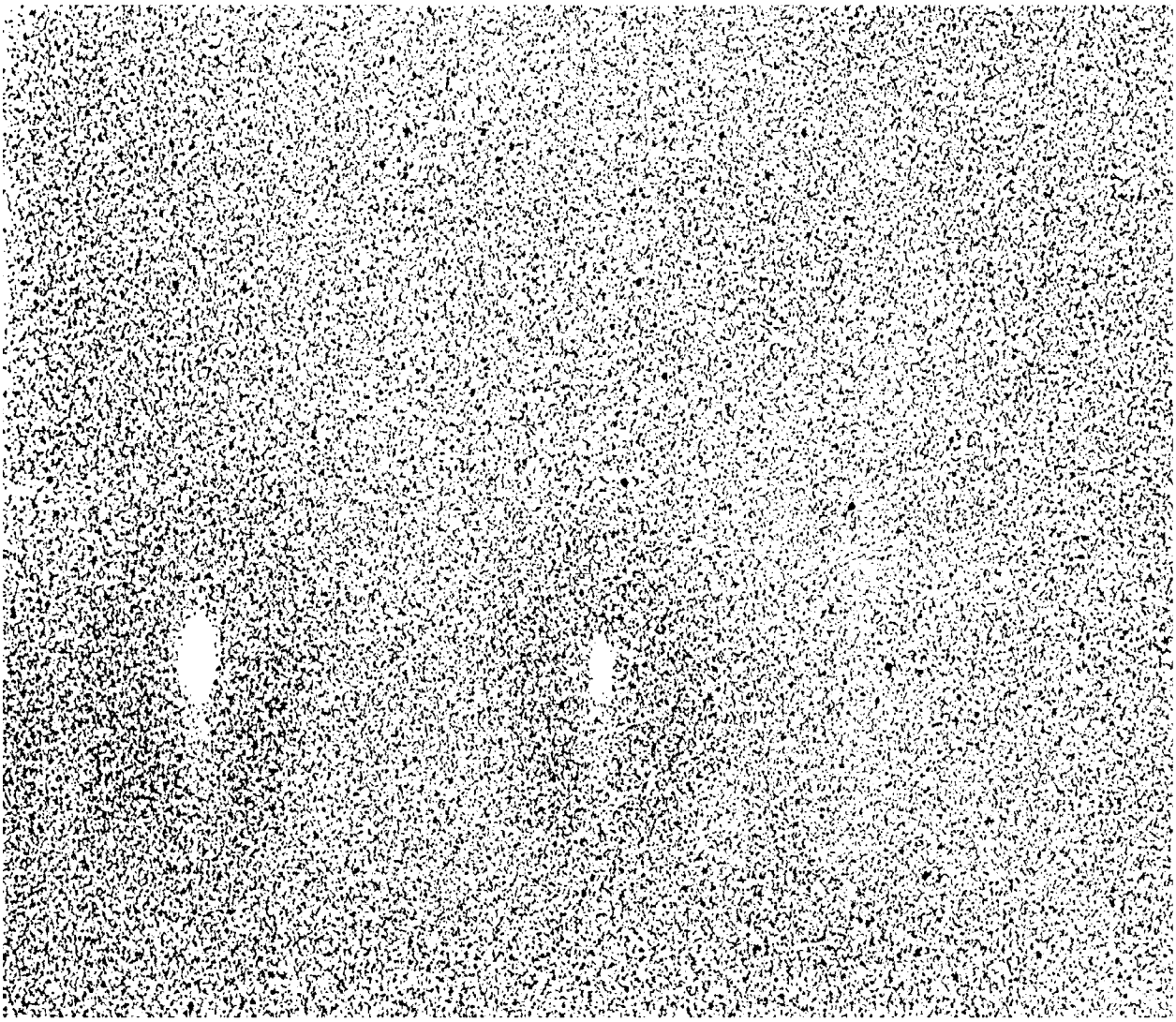

Fig. 1: Inhibitory white zones/or no zones of tomato four accessions leaf crude extract (5µl) compounds separated on TLC in hex/ether (80:20) and trichome anti fungal activity observed after the growth of *C. cucumerinum* on TLC plate

major contributor of the chemical components in the hexane and leaf wipe extracts. Khan and Snyder (1996) compared directly the anti fungal properties of *L. hirsutum* some accessions and *L. esculentum* leaf trichomes on both adaxial and abaxial surfaces on TLC against challenging fungi *C. Cucumerinum*. Lundgren *et al.*, (1985) also reported that one of the major volatile component from leaves of one accession of *L.hirsutum* was the sesquiterpene α. Santalen. Coates *et al.* (1988) reported the identification of three sesquiterpene carboxylic acids present in hexane extracts of leaves of *L. hirsutum* accession LA. 1777 as *α. santalen, β-bergamoten* and *α. bergamoten*. Several mono and sesquiterpenes in tomato leaf essential oils have been identified by GC/MS analysis (Juvik *et al*., 1988; Weston *et al*., 1989). Zafar *et al.* (2002) found that crude extracts of indigenous four different plant species leaves in hexane, chloroform, ethanol and water extracts showed some anti fungal activities against three fungi namely, *Rhizopous niger, F-chlamdosporum and Aspergillus niger*. Weston *et al.* (1989) found that by comparison of GC profiles, hexane leaflet washes were virtually identical with

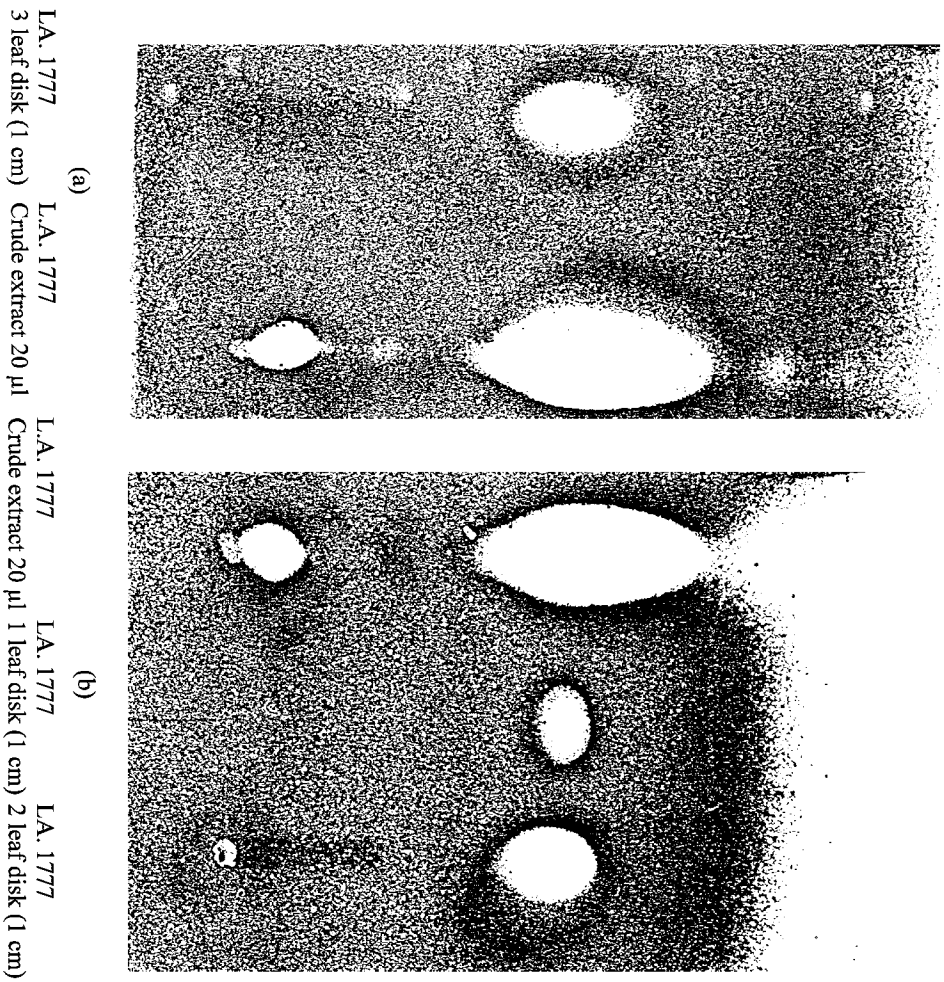

Fig. 2 (a) Inhibitory white zones/or no zones of tomato accessions LA. 1777 leaf crude extract and direct 3 leaf disk 1 cm (no treatment) compounds separated on TLC in hex/ether (80:20) and trichome anti fungal activity observed after the growth of *C. cucumerinum* on TLC plate.

(b) Inhibitory white zones/or no zones of tomato accessions LA. 1777 leaf crude extract and direct 1 and 2 leaf disk 1 cm (no treatment) compounds separated on TLC in hex/ether (80:20) and trichome anti fungal activity observed after the growth of *C. cucumerinum* on TLC plate.

secretions collected directly from type VI trichome glands. Thus, leaflet sample were considered to accurately represent trichome secretions. Juvik *et al.* (1988) conducted experiment to compare the chemical nature of leaf hexane extracts with that of trichome exudates, absorbent cotton was very lightly rubbed over the surface of attached leaves of green house-grown LA. 1777 plant. The cotton was then extracted in hexane and the extract then, gas chromatographed. The same peaks in roughly the same proportion were observed in chromatograms of the LA. 1777 hexane leaf extract and from the cotton wiped leaf tissue. Ch'erif *et al.* (1994) reported that different phenolic fractions were evaluated for fungi toxicity directly on chromatograms. Varying amounts were spotted on silica gel thin-layer chromatography (TLC) plates and developed with cyclohexane:ethyl acetate (1:1, v/v) or with chloroform:methanol (9:1, v/v). After drying for 2-3 h, the plates were sprayed with a conidial suspension of *Cladosporium cucumerinum* Ellis and Arth. The plates were incubated in a humid chamber for 72 h at room temperature, and zones of inhibition appeared as white spots on a grey background composed of *C. cucumerinum* spores and mycelium. Our study also revealed Fig. 2 (a and b) that the direct leaf disk and crude extract trichome exudates separated on TLC showed similar pattern of polar and non polar compounds and

fungi toxicity, with the total conformity with the preceding researchers that *Lycopersicon hirsutum* wild specie leaf surfaces trichomes consists of bio-chemical compounds inhibiting the growth of *C. cucumerinum in vitro*. However, our study has further looking in to the *L. hirsutum trichomes* anti fugal phenomenon, which was not studied before by other researchers, their work was related to insect repellency or toxicity. We also report that by separating the crude extract and touched leaf surface compounds on TLC, we found strong fungi toxicity only for third region compounds as shown in Fig. 1 and 2 (a and b). In conclusion it is stated that more research is needed to exploit the sources of naturally occurring fungi toxic compounds in *L. hirsutum* some accessions and other wild relatives of *L. esculentum* cultivated tomato.

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