of reduced and oxidized glutathione or by NADH in brain microsomes (table). Considering these facts, we suggest that the physiological amount of ascorbic acid present in the brain cytoplasm may be one of the factors protecting the membrane structures against peroxidative disintegration in the CNS.

Wills[8] explains the inhibitory effect of high ascorbic acid concentrations on lipid peroxide formation by assuming that, owing to their reductivity, they upset the balance between ferric and ferrous iron, essential for auto-oxidative processes. In the present experiments lipid peroxidation induced by ascorbic acid could be inhibited also by dehydroascorbic acid, showing that its structural properties rather than its reductivity are responsible for the inhibitory effect of large amounts of ascorbic acid. This, however, does not rule out the possibility that high concentrations of ascorbic acid, as an antioxidant agent, would inhibit the other types of peroxidation.

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Toxic substances produced by *Fusarium*. VII. Control of fusarial wilt of safflower by root exudates and extractives of *Ruellia tuberosa*

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Summary. Exudates and extractives of roots of *Ruellia tuberosa*, containing 2,6-dimethoxyquinone, acacetin and a C_{16}-quinone, have been shown to produce significant protective and curative actions against *Fusarium oxysporum*-incited wilt of safflower. The potentiality of the root extractives as a foliar fungicide is appraised.

Fusarium oxysporum f.sp. *carthami*-incited wilt of safflower in India was sometimes reported[2] to be as high as 25%. Another alarming aspect of the disease, reported recently[3-5], was the secretion of a number of mycotoxins by the fungus on safflower seeds in fields and during storage. Nonhost crops, e.g., maize, jawar, etc. were tried[6], from time to time, for reducing the fungal population in wilt-sick soil without achieving any great success. Recently, we reported[7] on the potentiality of 2,2',4-trihydroxybenzophenone as a foliar fungicide against this pathogen.

A remarkable observation was made by the present investigators while surveying the wilting of safflower in fields. The incidence of the disease was found to be very low in safflower plants fortuitously grown in the colony of *Ruellia tuberosa* L. (Acanthaceae). This observation prompted us to examine the effect of exudates and solvent extractives of roots of *R. tuberosa* on the most virulent strain of the pathogen, viz., *F. oxysporum* f.sp. *carthami* (IMI-186539)[2]. The details of these findings are reported in this communication.

Material and methods. Root portions of mature intact plants (65 g) of *R. tuberosa* were kept immersed (12 h) in sterile distilled water (100 ml). A portion of the aqueous solution (50 ml), containing the root exudates, was added to Richard's medium (100 ml). In the control, only distilled water (50 ml) was added. The 2 solutions were inoculated with the fungus and the mixtures were incubated at 21 °C for 7 days. The mycelial dry weight from the 2 cultures was determined. The mean of 3 experiments was recorded.

100 young plants of *R. tuberosa* were transplanted into 10 sterilized potting soil (ca. 2 kg, each). After acclimatization (4 weeks), the fungal mat (5 g), grown in Richard's medium, was added to each lot of potting soil. Subsequently, after 1 week, 10 surface-sterilized healthy safflower seeds were sown in each of the 10 lots of potting soil. The number of seedlings emerged with the fungal infection was recorded. The cotyledonary leaves of mildly affected safflower seedlings were sprayed with the aqueous solution of root exudates of *R. tuberosa*. After 2 weeks, the safflower seedlings were uprooted from the potting soil and a second batch of seeds was sown. For control experiments, susceptible safflower plants were used in place of *R. tuberosa* plants. Finally, the safflower seedlings and the *R. tuberosa* plants were uprooted and the fungal concentration in potting soil was determined by the Soil Smear technique[8] and by the Standard Soil Plate method[9].

In another set of experiments, aqueous sodium carbonate (1%) solution of petroleum ether (b.p. 60–80°) extracts of *R. tuberosa* roots (hereafter referred to as '*Ruellia* extracts'), mainly consisting of 3 chemical constituents in nearly equimolar quantities (average concentration ca. 1×10^{-3} M), was used for determining the antifungal activity. A portion (250 ml) of the aqueous sodium carbonate solution

was acidified with HCl and then extracted with chloroform. Column chromatography of the chloroform concentrate on silica gel (B.D.H., 60–120 mesh), using petroleum ether, benzene and different proportions of mixtures thereof, afforded 2,6-dimethoxybenzoquinone (23 mg), acacetin (15 mg) and a C_{16}-quinone (7 mg). The effect of the '*Ruellia* extracts' on the fungal hyphae was examined. The fungus was grown (48 h) in sterilized Richard's medium (150 ml) to which '*Ruellia* extracts' was added.

The extent of the mycelial growth after treatment with '*Ruellia* extracts' was quantitated. '*Ruellia* extracts' was added (1×10^{-3} M concentration of the contained chemical constituents) to Richard's medium (40 ml), which was inoculated with the fungus (ca. 1×10^6 spores/ml). The mixtures in the control and the treated groups were incubated at 21 °C for 7 days. Subsequently, the dry weight of the mycelial mat in the 2 groups was recorded.

10-day-old seedlings, grown on sterilized sand, were sprayed on the leaves with the '*Ruellia* extracts' 4 times at 12-h intervals. After 24 h of the last spray, the seedlings were uprooted and surface-sterilized (0.1% $HgCl_2$). Sections of leaves (1×0.1 cm), stems (0.2 cm, radius), and roots (0.1 cm) of the treated seedlings were placed on PDA (20 ml) plates seeded with the spore suspension (1 ml) of the fungus. The plates were incubated at 21 °C for 48 h and the inhibition zones were measured.

Results and discussion. The mycelial dry weights in the control and the root exudate-treated groups were 0.288 and 0.11 g, respectively. Only mild disease symptoms, as revealed by minute scattered spots on cotyledonary leaves, appeared in 30% of the seedlings in pots containing *R. tuberosa* plants. The remaining seedlings in these pots were disease-free. In the control groups, about 65% of the seedlings showed signs of severe afflictions. When the cotyledonary leaves of the mildly affected seedlings were sprayed with the aqueous solution of the root exudates, a significant recovery from the disease symptoms was observed after appearance of the first leaf. These leaves were almost normal in shape and size. The incidence of the disease in safflower was found to decline further in the second batch of seedlings in association of *R. tuberosa* plants. Thus, only 18% of the seedlings showed mild disease symptoms. In the control groups, over 80% of the seedlings were affected. The results indicate that the protective influence of the root exudates of *R. tuberosa* on safflower plants increases with the contact time of the former plants with the soil.

The rhizosphere samples in the control groups contained the fungus in the range of 150–250 chlamydospores, while those in the *R. tuberosa* groups contained only 30–50 chlamydospores.

The characterization of the 3 chemical entities, isolated from the '*Ruellia* extracts' was made on the basis of their physical and spectral properties. The middle petroleum ether eluates from the column chromatography afforded, on further preparative layer chromatopography, 2,6-dimethoxyquinone as orange needles, m.p. 248–250 °C, λ_{max}^{MeOH} nm (logε) 288 (4.44), ν_{max}^{KBr} 1698, 1642 cm^{-1}, m/e 168 (M, 88%), 138 (38), 69 (100). These properties are indistinguishable from those reported for the compound in the literature[10]. The compound was previously reported in a number of plants and fungi[11] but its biological activities have not been explored before.

The early benzene eluates afforded a yellow solid which crystallized from alcohol to give acacetin as yellow needles, m.p. 254–255 °C, λ_{max}^{MeOH} nm (logε) 270 (4.37), 305 sh (3.98), 325 (4.50), m/e 284 (M^+, 100%), 283 (8), 256 (4), 153 (1), 152 (5), 132 (11). Its identity was further confirmed by direct comparison (m.p., mixed m.p., co-TLC) with an authentic sample.

The later benzene eluates yielded a C_{16}-quinone ($C_{16}H_{12}O_6$) as a red gum, R_f 0.32 (benzene-acetic acid, 100:4, TLC), λ_{max}^{MeOH} nm (logε) 288 (4.36), 305–310 (3.88), ν_{max}^{KBr} 1695, 1668, 1645, 1610, 1595 cm^{-1}, m/e 300 (M^+, 32%), 272 (8), 181 (88), 147 (5), 119 (100). The compound could be reduced to a yellow chalcone by SO_2 and the latter suffered autoxidation to the red quinone. These properties are closely similar to those of carthamone[12], a red glucosyloxychalconoquinone previously encountered in safflower.

The hyphal cells were lysed within 48 h of addition of the '*Ruellia* extracts'. The mycelia became black, the protoplasts were contracted and detached from the cell wall and had collected at one corner or in the middle of the cells. Similar observations were recently reported[13] when the fungus was treated with a glucoxanthone, mangiferin.

The mycelial weight (in g±SEM) in the control and the '*Ruellia* extracts'-treated groups were 350 ± 0.002 and 0.115 ± 0.0012, respectively ($p < 0.01$).

The '*Ruellia* extracts' when sprayed on safflower leaves, around the sections of the leaves, stems and roots a clear inhibition zone of the fungus was observed. The maximum inhibition zone (0.55 cm) was observed around the leaf section. The inhibition zone around the root section was minimum but was also appreciable (0.28 cm). The results indicate that the fungicidal principle contained in the '*Ruellia* extracts' is capable of translocation from leaves to roots of safflower.

Resistance is obviously a complicated phenomenon not dependent on any single property. It presumably occurs when a delicate balance of a number of interacting factors, which include antifungal agents preformed, induced and/or introduced, is tilted in favour of the host. The increase in the concentration of carthamone[12], a glucosyloxychalconoquinone of safflower, in response to the fungal infection and the concomitant control of the wilting disease has earned[14] for it billing 'phytoalexin' of safflower. It would now seem likely that the actions of the quinone(s) of *R. tuberosa* as such, or as synergist(s) to carthamone, could be responsible for the defence of safflower (*Carthamus tinctorius* L.) against the wilting disease.

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