radicals in aqueous phase, more effective than any other endogenous antioxidant[12]. Alloxan induced LPO in rat erythrocytes, however, such an effect was not observed in presence of AA. Glucose in physiological amounts showed no significant change in erythrocyte LPO with or without AA. However, high amounts of glucose have been shown to produce toxic oxygen species in presence of transition metals[13].

Alloxan with or without glucose increased oxidative reaction and generation of oxygen-free radicals. These effects of alloxan were observed in absence of AA. In presence of AA no significant increase in erythrocyte LPO was observed in alloxan-treated erythrocytes with or without glucose, suggesting the role of O_2^- anion in the induction of alloxan-induced LPO in erythrocytes. AA acts as scavenger of the free oxygen species, thus lowering LPO in erythrocytes treated with AA.

The increase in SOD and CAT activity due to glucose in absence of AA may be an attempt by erythrocytes to counteract any oxidative change. However, in presence of AA, no significant change was observed in erythrocyte SOD and CAT activities or in LPO, which indicate that free radical species are effectively neutralized by AA.

Alloxan due to the production of free radical species such as O_2^- inhibited SOD activity which may be one of the reasons for increased LPO in erythrocytes. The increase in CAT activity may be due to increased production of H_2O_2 in presence of alloxan. As the changes in SOD and CAT activity in alloxan-treated erythrocytes recover towards normal in presence of AA, it is suggested that these enzymes are modified in presence of alloxan and the action is mitigated by AA. Reactive oxygen species (ROS) not only induce LPO but also modify enzymes. The involvement of ROS in the modification of protein kinase C has been suggested to be an effective on/off signal mechanism to influence cellular events[14]. ROS has been shown to induce signalling including activation of protein kinase[14], induce protein phosphorylation[15] and also act as second messenger for the expression of genes involved in the immune response[16]. It is probable that ROS may modify reactive oxygen scavenger enzymes and in presence of AA may show dual activation–inactivation of these enzymes.

It is concluded that alloxan due to the production of free radical species increased LPO and altered antioxidant enzymes in erythrocytes. The treatment with glucose in physiological amounts showed no effect on erythrocyte LPO but increased SOD and CAT in glucose-treated erythrocytes in absence of AA. However, no significant changes were observed in LPO, SOD and CAT activities in erythrocytes treated with glucose in presence of AA. Alloxan and glucose treatment in absence of AA increased LPO and decreased SOD and CAT activities in erythrocytes. None of these changes was observed in presence of AA. Thus, it may be one of the processes, how the cells respond to and mitigate the ill effects of reactive oxygen intermediates engendered in biological systems by diverse processes.

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Plant extracts: A non-chemical approach to control *Fusarium* diseases of mulberry

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Leaf extracts of *Azadirachta indica*, *Calotropis gigantea*, *Eucalyptus* sp., *Parthenium hysterophorous* and *Pongamia pinnata* were evaluated for their antifungal activity against *Fusarium pallidoroseum* and *F. moniliforme* var. *intermedium* causing leaf blights, and *F. oxysporum* causing leaf spot diseases in mulberry (*Morus alba* L.). Leaf extract (1 : 5) of *P. pinnata* was highly fungitoxic to *F. pallidoroseum* and *F. moniliforme* var. *intermedium* inhibiting their mycelial growth in plates by 78.2% and 84.3%, respectively; whereas *C. gigantea* and *A. indica* were most effective

against *F. oxysporum* inhibiting its mycelial growth by 78.5% and 73.2%, respectively. Under greenhouse conditions, aqueous leaf extract (25%) of *P. pinnata* reduced the incidence of leaf blights caused by *Fusarium pallidoroseum* and *F. moniliforme* var. *intermedium* by 63.6% and 67.1%, respectively; whereas in case of leaf spot caused by *F. oxysporum*, *C. gigantea* and *A. indica* both were effective in reducing the leaf spot incidence by 60.2% and 57.2%, respectively.

In mulberry, *Fusarium* diseases are the most destructive ones which are prevalent in all the southern states of the country where sericulture is generally practised. These diseases are observed in all the seasons with varying degree of disease incidence (3.0–19.5%) which reduce the leaf yield and its nutritive value, and make mulberry leaves unsuitable for silkworm feeding[1–3]. Therefore, keeping in view the seriousness of *Fusarium* diseases in mulberry, and possible harmful effects of chemical fungicides on silkworm growth and development, there is a need to develop a non-chemical, eco-friendly method for the control of these diseases.

In the recent past, the angiospermic plants are proved to be a useful source of fungitoxic substances that are rather harmless compared to synthetic chemical fungicides which often impose undesirable side effects. Several plants have been reported to possess substances which are toxic to microbial pathogens and serve as protective barriers to infection[4]. The present investigation was, therefore, undertaken to test the efficacy of leaf extracts of *Azadirachta indica* A. Juss., *Calotropis gigantea* (L.) R. Br., *Eucalyptus* sp., *Parthenium hysterophorus* L. and *Pongamia pinnata* (L.) Pierre against *Fusarium* species, viz. *F. pallidoroseum* (Cooke) Sacc., *F. moniliforme* Sheldon var. *intermedium* Neish & Leggett and *F. oxysporum* Schlecht. causing leaf blights and leaf spot diseases in mulberry[2,3].

Twenty-five grams of fresh leaves of each plant species, viz. *Azadirachta indica*, *Calotropis gigantea*, *Eucalyptus* sp., *Parthenium hysterophorus* and *Pongamia pinnata* (washed well 2–3 times with tap water and once with distilled water) were ground in 100 ml of sterile distilled water. The macerate was squeezed through double-layered muslin cloth and centrifuged at 5,000 rpm for 20 min. The supernatant was filtered through Whatman No. 1 filter paper and then sterilized by passing through the Seitz filter (G5). The extract (25%) thus obtained was used for the *in vitro* experiments. For *in vivo* greenhouse experiments, the macerate obtained after squeezing through double-layered muslin cloth (25% aqueous leaf extract) was directly used for spraying plants.

For evaluation of antifungal effect of leaf extracts on mycelial growth of *Fusarium* species in plates, potato dextrose agar medium was amended with filter-sterilized fresh leaf extract (25%) to get dilution of 1 : 5, 1 : 10, 1 : 20 and 1 : 30 (extract : medium). The medium devoid of leaf extract served as control. The medium was poured into Petri plates (90 × 17 mm) at 15 ml/plate. The plates were inoculated centrally with a 4 mm mycelial disk (cut from the edge of 5-day-old actively growing colonies) of each species of *Fusarium*. Four replicates were maintained for each dilution and control. The whole set of experiment was incubated at 28 ± 2°C and mean radial growth of fungal colony was recorded after a week. The fungitoxic efficacy of leaf extract was determined by comparing the radial growth in treatment (T) with the control (C). The inhibition percentage (I) was calculated by following the formula[5]: $[I = (C - T/C) \times 100]$.

Leaf extracts found effectively inhibiting the growth of *Fusarium* species on plates, were tested *in vivo* under greenhouse conditions. Extracts of *P. pinnata*, *C. gigantea* and *A. indica* were tested against leaf blights caused by *F. pallidoroseum* and *F. moniliforme* var. *intermedium*, whereas *C. gigantea* and *A. indica* were tested against leaf spot caused by *F. oxysporum*.

Mulberry (*Morus alba* L.) var. Kanva 2 plants, susceptible to *Fusarium* species tested, were raised from healthy cuttings and grown in earthen pots (40 cm) in a greenhouse at 28 ± 2°C with relative humidity of 75%. Three sets of required number of potted plants (2-month-old) were inoculated separately with *F. pallidoroseum*, *F. moniliforme* var. *intermedium* and *F. oxysporum* by atomizing their freshly prepared uncontaminated spore suspensions (1×10^6 conidia/ml) on to the leaves of plants. Inoculated plants were covered individually with a moist polythene bag for 24 h. After 72 h of inoculation, aqueous leaf extracts (25%) of *P. pinnata*, *C. gigantea* and *A. indica* were sprayed separately on to the leaves of plants inoculated with *F. pallidoroseum* and *F. moniliforme* var. *intermedium*; whereas extract of *C. gigantea* and *A. indica* were sprayed on to the leaves of plants inoculated with *F. oxysporum*. The extracts were sprayed twice at an interval of one week. Three replications of 5 plants each (total 15 plants) were maintained for each extract and *Fusarium* species, and the same number of plants from each *Fusarium*-inoculated set were sprayed with sterile distilled water which served as control. All the replications, including control, were arranged in a randomized block design. Disease intensity was recorded in treated and control plants after 30 days of second spray. From the observations, the per cent disease incidence (PDI) values (severity of disease) were calculated according to the FAO formula for plant disease assessment in different grades[6].

$$\text{PDI} = \frac{\text{Sum of numerical values}}{\text{Total number of leaves observed} \times \text{Maximum grading (5)}} \times 100.$$

The sum of numerical values was obtained by mul-

tiplying the number of leaves observed in a particular grade with their respective grading as given below: 1 = No infection; 2 = up to 5% of leaf area affected; 3 = 6–25% of leaf area affected; 4 = 26–50% leaf area affected; and 5 = 51–100% leaf area affected.

From the PDI in control (PC) and treatment (PT), the per cent disease control (PDC) was calculated by following the formula: [PDC = {(PC – PT)/PC} × 100].

The per cent inhibition data (Table 1) were statistically analysed by subjecting them to ANOVA, whereas qualitative PDI data were analysed by following Mann–Whitney *U* test.

In order to confirm the results, each experiment was repeated three times.

Significant reduction ($p \geq 0.05$) in radial growth of *F. pallidoroseum*, *F. moniliforme* var. *intermedium* and *F. oxysporum* was observed with leaf extracts of *A. indica*, *C. gigantea*, *Eucalyptus* sp. and *P. pinnata*. However, extracts of *P. hysterophorus* stimulated the growth of all *Fusarium* species, even at a lower concentration of 1 : 30. The inhibition effect of leaf extracts was directly correlated with their concentration which increased with the concentration of extracts. Both the leaf blight causing *F. pallidoroseum* and *F. moniliforme* var. *intermedium* showed similar trends of inhibition in their mycelial growth, however, *F. oxysporum* causing leaf spot disease differed from these two species in responding to leaf extracts.

Leaf extracts of *P. pinnata* was highly fungitoxic at all concentrations to *F. pallidoroseum* and *F. moniliforme* var. *intermedium* inhibiting their radial growth in plates by 63.1–84.3%; whereas *Eucalyptus* sp. was least effective to both *Fusarium* species, even at a high concentration of 1 : 5 as it showed only 30.5% and 22.0% growth inhibition, respectively. *C. gigantea* was moderately effective against both the leaf blights causing *Fusarium* species inhibiting 50.3–71.8% growth (Table 1 and Figure 1).

Leaf extracts of *C. gigantea* and *A. indica* were found to be most effective at all concentrations against *F. oxysporum* inhibiting 58.5–78.5% and 59.7–73.2% growth, respectively. *P. pinnata* extract, though highly fungitoxic to both the leaf blight causing *F. pallidoroseum* and *F. moniliforme* var. *intermedium*, exhibited least inhibition potential (0.8–12.2%) against leaf spot causing *F. oxysporum* (Table 1 and Figure 1).

Leaf extracts of *A. indica* and *P. pinnata* have earlier been reported inhibiting the mycelial growth of various

Table 1. Fungitoxic potential of leaf extracts against *Fusarium* species *in vitro*

Plant species	Conc. of extract	Inhibition/stimulation (+) percentage		
		F. pallidoroseum	*F. moniliforme* var. *intermedium*	*F. oxysporum*
Azadirachta indica	1 : 5	44.9	61.2	73.2
	1 : 10	38.5	58.2	69.4
	1 : 20	27.5	52.9	65.3
	1 : 30	24.7	50.9	59.7
		{0.12}	{0.04}	{0.03}
Calotropis gigantea	1 : 5	68.3	71.8	78.5
	1 : 10	62.6	67.6	73.4
	1 : 20	55.3	63.1	66.9
	1 : 30	50.3	58.5	58.5
		{0.07}	{0.03}	{0.03}
Eucalyptus sp.	1 : 5	30.5	22.0	21.1
	1 : 10	13.1	10.5	11.8
	1 : 20	6.3	5.5	4.4
	1 : 30	1.8	1.7	1.1
		{0.07}	{0.04}	{0.02}
Pongamia pinnata	1 : 5	78.2	84.3	12.2
	1 : 10	74.1	80.9	4.8
	1 : 20	68.8	72.8	2.0
	1 : 30	63.1	68.9	0.8
		{0.06}	{0.06}	{0.03}
Parthenium hysterophorus	1 : 5	+ 20.0	+ 23.9	+ 11.5
	1 : 10	+ 10.2	+ 15.3	+ 7.1
	1 : 20	+ 4.9	+ 6.3	+ 4.1
	1 : 30	+ 2.4	+ 3.8	+ 1.2
		{0.09}	{0.03}	{0.02}
C.D. {≥0.05} between extracts.		0.03	0.15	0.01

C.D. {≥0.05} between concentration of individual extract (in parentheses).

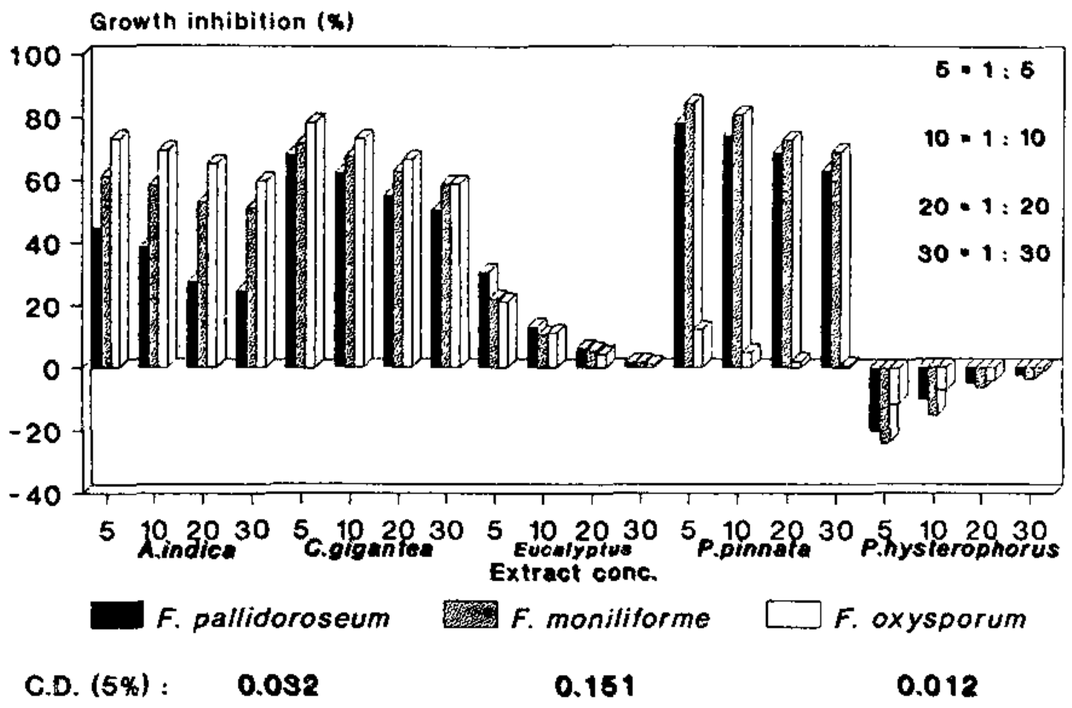

Figure 1. Fungitoxic potential of leaf extracts against *Fusarium* species *in vitro*.

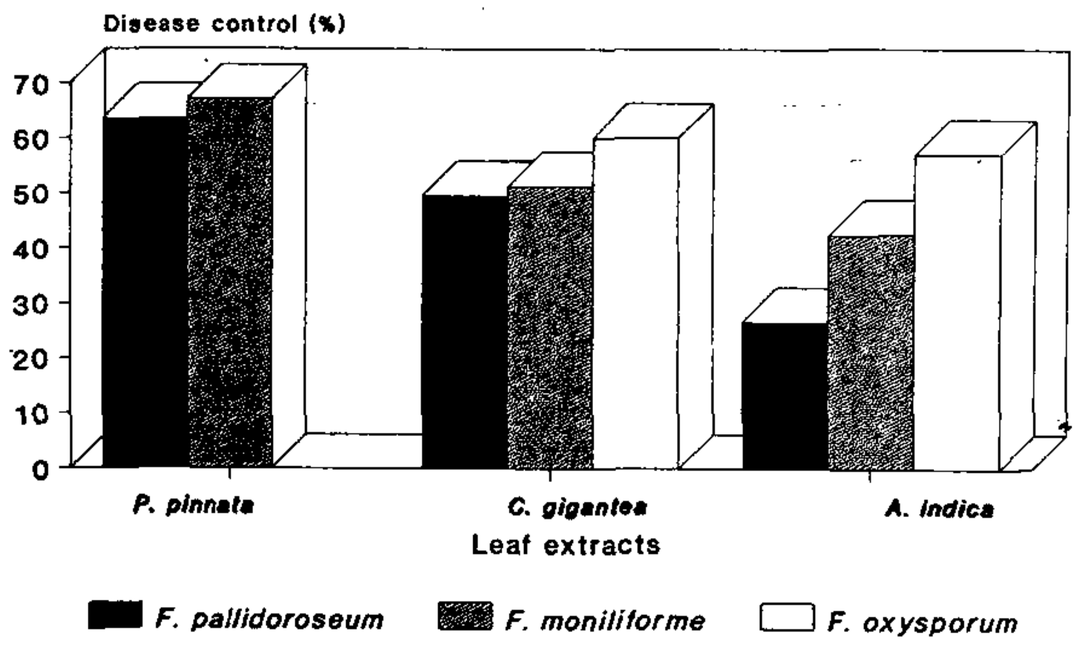

Figure 2. Efficacy of leaf extracts in controlling the *Fusarium* diseases of mulberry.

Fusarium species[7,8], which is in agreement with the present findings. Leaf extracts of *Eucalyptus* sp. and *C. gigantea* have also been known for their antifungal activity against various fungal pathogens[9,10]. However, these extracts have not been studied against any species of *Fusarium*. The inhibitory effect of all these leaf extracts on mycelial growth might be attributed to the presence of antifungal ingredients in them[8,9,11]. *P. hysterophorus* leaf extract, though reported for its antifungal properties against *F. oxysporum* and some other fungal pathogens[12,13], is found stimulatory to *Fusarium* species infecting mulberry. It may be due to the variations in response of different *Fusarium* species and formae speciales to the leaf extracts[4].

In greenhouse experiment, leaf extracts of *P. pinnata*, *C. gigantea* and *A. indica* were proved to be fungicidal as these extracts significantly controlled the leaf blights and leaf spot diseases caused by *Fusarium* species in mulberry. Aqueous extract (25%) obtained from the fresh leaves of *P. pinnata* showed better fungicidal potential by reducing the disease severity (PDI) of *F. pallidoroseum* and *F. moniliforme* var. *intermedium* leaf blights by 63.6% and 67.1%, respectively. In the case of leaf spot caused by *F. oxysporum*, leaf extracts of *C. gigantea* and *A. indica* were able to control the disease severity by 60.2% and 57.2%, respectively (Figure 2). These results are in agreement with the earlier observations[10], particularly in case of *C. gigantea* and *A. indica* which have been reported to reduce the disease severity of leaf spot caused by *Cercospora moricola* in mulberry. However, contrary to the earlier report[10], in the present study the leaf extract of *P. pinnata* is highly effective in controlling the leaf blights caused by *F. pallidoroseum* and *F. moniliforme* var. *intermedium* in mulberry. This is the first report on evaluation of leaf extracts for the control of *Fusarium* diseases of mulberry under realistic conditions in greenhouse.

All the leaf extracts tested were found to be fungicidal, but non-phytotoxic in nature. Thus, these extracts can be exploited for the control of *Fusarium* diseases of mulberry.

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