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Integrated management of late leaf spot and rust diseases of groundnut (*Arachis hypogaea* L.) with *Prosopis juliflora* leaf extract and chlorothalonil

G. KRISHNA KISHORE & SURESH PANDE

Pathology, International Crops Research Institute for the Semi-Arid Tropics, Patancheru, AP, India

Abstract
Late leaf spot (LLS, *Phaeoisariopsis personata*) and rust (*Puccinia arachidis*) are the two major biotic constraints of groundnut (*Arachis hypogaea* L.) of global importance. To identify economic and eco-friendly disease management options, we evaluated extracts of 38 plant spp. of 23 families, for their antifungal activity. Aqueous leaf extracts (20%, w/v) of *Prosopis juliflora* and *Lycopersicon esculentum* completely inhibited the *in vitro* germination of *P. personata* and *P. arachidis*, and extracts of *Achras sapota*, *Cyamposis tetragonolobus*, *Piper betle* and *Tagetus patula* were inhibitory by >95%. In the greenhouse, the severity of LLS and rust corresponded with the time interval between the foliar application of leaf extract and fungal inoculum. Extract of *P. juliflora* (2%, w/v) in simultaneous application reduced the lesion frequency of LLS and rust by ~75%, and 35.7% and 50.7% in a prophylactic spray of 96 h before the pathogen inoculation. The extract had no effect on the phenolic content of groundnut leaves both during LLS and rust infections. In the field, *P. juliflora* extract applied four times at 15-day intervals, was effective against LLS and rust up to 95 days after sowing (d.a.s.). Foliar application of *P. juliflora* extract at 45, 75 and 90 d.a.s. and chlorothalonil at 60 d.a.s. effectively reduced foliar diseases severity and increased the pod yields by 81 – 98%. This study identified *P. juliflora* extract as a significant component for the integrated management of groundnut foliar diseases.

Keywords: *Antifungal, integrated disease management, natural fungicide, peanut*

1. Introduction

Groundnut (*Arachis hypogaea* L.) is a major food and cash crop in several of the semi-arid tropics (SAT) of Asia and Africa. Groundnut production is limited by late leaf spot (LLS) caused by *Phaeoisariopsis personata* (Berk. & Curt.) v. Arx and rust caused by *Puccinia arachidis* Speg., the two major biotic constraints of global significance (Subrahmanyam et al. 1995). Combined infection of LLS and rust is quite frequent and results in pod yield losses of >70%, if not managed (Savary and Zadoks 1992). The deployment of host plant resistance for LLS and rust management is limited by the absence of desirable levels of disease resistance coupled with agronomic traits such as seed shape, testa colour and pod filling, in cultivated groundnut. Chemical management of LLS and rust needs repeated application (3 – 4 times) of the fungicides. The cost of fungicides is often prohibitive in much of the SAT, as yields are often restricted by drought. As a result, there is a need for better economic management of LLS and rust, and we explored for alternate fungicides of plant origin.

Several higher plants are known to possess antifungal metabolites and the exploration of other plants continues in search of new sources of cost-effective fungicides. Non-phytotoxicity and biodegradability are additional advantages of fungicides of plant origin (Cutler 1988). The fungicidal activity of plant extracts has been used in the control of foliar, soil-borne and post harvest fungal diseases. The foliar application of plant extracts restricted the development of Alternaria blight of sunflower (Chattopadhyay 1999), powdery mildew of cucumber (Daayf et al. 2000) and ergot of sorghum (Singh and Navi 2000). Soil amendment of plant extracts or their formulations reduced the soil population density of *Phytophthora* sp. (Bowers and Locke 2004) and *Fusarium oxysporum* (Bowers and Locke 2000) in different plant pathogen systems. Active metabolites of a few plant extracts have been isolated (Grayer and Harborne 1994) and their efficacy against phytopathogenic fungi (Newman et al. 1999) was determined.

In a previous study, we demonstrated the antifungal activity of aqueous leaf extracts of *Datura metel* and *Lawsonia inermis* against *P. personata* (Kishore et al. 2001). In a search of further effective and safe fungicides, we continued to determine the antifungal activity of 38 additional plant species against *P. personata* and *P. arachidis*. *Prosopis juliflora* leaf extract tested for control of LLS and rust in

Correspondence: G. Krishna Kishore, Plant Gene Resources of Canada, Saskatoon Research Centre, 107 Science Place, Saskatoon 57N 0X2, Canada. Tel: 001 306 956 7689. E-mail: krishnakishore_gali@yahoo.co.in

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greenhouse and field, and its effect on the phenol metabolism of groundnut was studied. The long-term goal of this study is to evaluate plant extract(s) as important component of integrated disease management (IDM) of LLS and rust.

2. Materials and methods

2.1. Fungal isolates

Single lesion isolates of *P. personata* and *P. arachidis* from LLS and rust infected groundnut plants were multiplied on groundnut cv. TMV 2 using a detached leaf technique (Subrahmanyam et al. 1983).

2.2. Preparation of leaf extracts

Aqueous leaf extracts of 38 plant species belonging to 23 families (Table I) were used in this study. Young, healthy leaves of each plant spp. were washed thoroughly with running tap water followed by sterile distilled water (SDW). Twenty grams of the leaves were homogenized in 30 ml of SDW using a waring blendor, filtered through cheesecloth and centrifuged at 10,000 rpm for 10 min at room temperature. The supernatant was used as a crude extract in different experiments and filter sterilized to test the *in vitro* antifungal activity.

2.3. Preparation of inoculum

Freshly collected spores of *P. personata* and *P. arachidis* were suspended separately in SDW containing 0.01% (v/v) Tween 80 as a wetting agent. The spore concentration was determined using a hemocytometer and diluted with SDW.

2.4. In vitro *antifungal activity*

The effect of leaf extracts on the *in vitro* germination of conidia of *P. personata* and urediniospores of *P. arachidis* was quantified. Fifty μl of each leaf extract (40%, w/v) with SDW as control were mixed with equal volume of conidia or urediniospore suspension (5×10^4 spores ml^{-1}) on a cavity slide. The slides were placed in a humid chamber and incubated in dark at $24 \pm 1^{\circ}C$, and observed for germination of conidia and urediniospores after 24 and 8 h of incubation. In each treatment 100 spores were observed and the experiment was repeated twice with three replications. Antifungal activity of each extract was expressed as percentage inhibition of conidial or urediniospore germination with respect to control. The experiment was repeated with 5, 2 and 1% (w/v) final concentrations of six selected leaf extracts.

2.5. Greenhouse evaluation of leaf extracts

Aqueous leaf extracts (2%, w/v) of *Prosopis juliflora* and *Lycopersicon esculentum*, selected for their high antifungal activity at low concentrations, were tested for control of LLS and rust in greenhouse. Thirty-day-old groundnut plants of cv. TMV 2 (highly susceptible to LLS and rust) grown in the greenhouse were used in these experiments. The two extracts, with SDW as control, were applied as a foliar spray at 96, 48 and 24 h and 10 min before the pathogen inoculation. Artificial inoculation of LLS and rust was done by foliar application of *P. personata* and *P. arachidis* (2×10^4 spores ml^{-1}) inoculum using a hand-operated atomizer. The inoculated plants were maintained at a temperature of $24 \pm 2^{\circ}C$. Following pathogen inoculation, alternate wet (16 h) and dry (8 h) periods of leaf wetness were provided by shifting the pots between dew chambers (Clifford 1973) and greenhouse for up to 8 days after inoculation (d.a.i.). In each plant, lesion frequency (LF, number of lesions cm^{-2} leaf area) on third or fourth leaf from the top tagged at the time of inoculation, and disease score on a 1–9 rating scale were measured 15 and 30 d.a.i. The experiment was repeated twice with four replications and 20 plants in each treatment.

2.6. *Effect of* P. juliflora *extract on phenol metabolism of groundnut*

The effect of foliar application of *P. juliflora* leaf extract on the total phenolic content of groundnut leaves was determined to understand its role in activation of groundnut defense responses. The leaf extract (2% w/v) was applied as a foliar spray on to 30-day-old plants of cv. TMV 2. After 24 h, the treated plants were challenge inoculated with *P. personata* or *P. arachidis* using SDW as control. The third or fourth leaf from the top was excised from the inoculated plants at regular intervals up to 11 d.a.i., and used for estimation of total phenols. The experiment was repeated twice with three replications of each treatment.

Total phenolics were determined using the Folin–Ciocalteau reagent (Singleton and Rossi 1965). To 1 g of leaf tissue, 5–8 ml of 80% ethanol was added and incubated in a water bath at $80^{\circ}C$ for 10 min. The solution was filtered and the filtrate was centrifuged at 10,000 rpm for 15 min. The volume of the supernatant was made to 5 ml with ethanol. To 1 ml of the extract, 1 ml of Folin–Ciocalteau reagent and 2 ml of 20% Na_2CO_3 solution were added and incubated in a boiling water bath for 1 min. The reaction mixture was cooled immediately, diluted to 25 ml with distilled water and the absorbance was measured at 650 nm using catechol as standard. The results were expressed as μg catechol g^{-1} of fresh weight.

2.7. *Field evaluation of* P. juliflora *leaf extract*

Field evaluation of *P. juliflora* leaf extract in combination with chlorothalonil (kavach®) for

Table I. *In vitro* antifungal activity of aqueous leaf extracts of different plant species against *Phaeoisariopsis personata* and *Puccinia arachidis*.

S. no.	Plant spp.	Family	Percentage spore germination	
			P. personata	*P. arachidis*
1	*Acacia catechu* (L.f.) Willd.	Fabaceae	45.4 (48)	3.1 (97)
2	*Achras sapota* L.	Sapotaceae	0 (100.0)	3.0 (97)
3	*Allium cepa* L.	Amaryllidaceae	3.6 (96)	4.7 (95)
4	*Annona squamosa* L.	Annonaceae	47.4 (46)	28.3 (70)
5	*Artocarpus heterophyllus* Lam.	Moraceae	56.7 (35)	5.4 (94)
6	*Bougainvillea spectabilis* Willd.	Nyctaginaceae	61.0 (31)	5.2 (94)
7	*Capsicum annum* L.	Solanaceae	19.9 (77)	15.8 (83)
8	*Carica papaya* L.	Acanthaceae	6.8 (92)	42.4 (54)
9	*Carthamus tinctorius* L.	Asteraceae	8.8 (90)	31.6 (66)
10	*Citrus limon* (L.) Burm.	Rutaceae	27.8 (68)	33.9 (64)
11	*Citrus sinensis* (L.) Osbeck	Rutaceae	34.8 (60)	20.6 (78)
12	*Corchorus capsularis* L.	Tiliaceae	79.2 (10)	24.4 (74)
13	*Coriandrum sativum* L.	Apiaceae	37.4 (57)	7.6 (92)
14	*Curcuma longa* L.	Zingiberaceae	40.0 (54)	2.2 (98)
15	*Cyamopsis tetragonolobus* (L.) Taub	Fabaceae	0 (100)	2.7 (97)
16	*Emblica officinalis* L.	Euphorbiaceae	62.6 (29)	5.0 (95)
17	*Ficus bengalensis* L.	Moraceae	47.9 (45)	6.6 (93)
18	*Helianthus annuus* L.	Asteraceae	50.1 (43)	16.0 (83)
19	*Leucaena leucocephala* (Lam.) de wit	Fabaceae	68.7 (22)	88.4 (5)
20	*Lycopersicon esculentum* Mill.	Solanaceae	0 (100)	0 (100)
21	*Mangifera indica* L.	Anacardiaceae	74.8 (15)	36.2 (61)
22	*Mentha arvensis* L.	Lamiaceae	55.9 (36)	33.4 (64)
23	*Momordica charantia* L.	Cucurbitaceae	44.6 (49)	7.0 (92)
24	*Moringa oleifera* Lam.	Moringaceae	44.5 (49)	8.8 (91)
25	*Nelumbo nucifera* Gaertn.	Nelumbonaceae	72.5 (17)	21.3 (77)
26	*Piper betle* L.	Piperaceae	2.4 (97)	1.9 (98)
27	*Piper nigrum* L.	Piperaceae	30.4 (65)	18.4 (80)
28	*Prosopis juliflora* DC.	Mimosaceae	0 (100)	0 (100)
29	*Psidium guajava* L.	Myrtaceae	63.1 (28)	1.1 (99)
30	*Punica granatum* L.	Punicaceae	55.9 (36)	16.1 (83)
31	*Ricinus communis* L.	Euphorbiaceae	37.8 (57)	37.7 (59)
32	*Syzigium cumin* L.	Myrtaceae	47.9 (45)	15.6 (83)
33	*Tagetus patula* L.	Asteraceae	4.8 (95)	0 (100)
34	*Tamarindus indica* L.	Caesalpiniaceae	76.3 (13)	70.0 (25)
35	*Terminalia catappa* L.	Combretaceae	69.6 (21)	39.3 (58)
36	*Trachyspermum ammi* (L.) Sprague	Apiaceae	22.8 (74)	2.1 (98)
37	*Trigonella foenum-graecum* L.	Fabaceae	28.2 (68)	6.6 (93)
38	*Zingiber officinale* Rosc.	Zingiberaceae	70.9 (19)	7.2 (92)
39	Control		87.8	93.0
	SE_m		2.7	2.2
	F-probability		<0.001	<0.001
	degrees of freedom		38	38

The values are the mean of nine replications in three sets of experiments. Values presented in parenthesis represent the percentage inhibition of spore germination in individual treatment with respect to control.

control of LLS and rust of groundnut was carried out during 2002 and 2003 rainy seasons in farm fields of the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, India. The experiment was conducted in a completely randomized block design with three replications. In each treatment, six rows each of 9 m length were considered as a single replication. On either side of the test rows of each treatment, one row of TMV 2 as infector row was planted at 15 days in advance.

The experimental fields were routinely used for groundnut foliar diseases screening for the past 25 years so that local inoculum levels would be high. Additionally, at 40–45 d.a.s. the infector rows were inoculated with a suspension of conidia and urediniospores (3×10^4 spores ml^{-1}), previously collected from infected plants in greenhouse and stored at 4°C for a maximum period of 30 days. Pathogen inoculation was done at 18:00 h and prior to inoculation the field was furrow irrigated. During the subsequent 10 days, the field was irrigated with overhead sprinklers for 30 min before sunset. Starting from 20 days after pathogen inoculation, the sprinkler irrigation was continued till 90 d.a.s. on rain-free days to facilitate secondary infection cycles (Subrahmanyam et al. 1995).

The following treatments applied as a foliar spray were evaluated for control of LLS and rust: (a) aqueous leaf extract (2%, w/v) of *P. juliflora* at 45, 60, 75 and 90 d.a.s., (b) *P. juliflora* leaf extract at 60, 75 and 90 d.a.s., and chlorothalonil ($2\,g\,l^{-1}$) at 45 d.a.s., (c) *P. juliflora* leaf extract at 45, 75 and 90 d.a.s., and chlorothalonil at 60 d.a.s., (d) *P. juliflora* leaf extract at 40, 60 and 90 d.a.s., and chlorothalonil at 75 d.a.s., and (e) chlorothalonil at 45, 60, 75 and 90 d.a.s., and (f) tap water as control at 45, 60, 75 and 90 d.a.s.

In all the experimental plots, foliar diseases severity was scored at 10-day intervals from 55 to 105 d.a.s. Quantification of combined infection of LLS and rust was based on a 1–9 rating scale (1 = healthy plants and 9 = plants severely affected and 50–100% leaves withered or defoliated) (Subrahmanyam et al. 1995).

2.8. Statistical analysis

Data from different experiments were subjected to ANOVA, one- or two-way ANOVA in randomized block design (Payne 2002). Mean values of different treatments were compared using least significant difference (LSD) at $P = 0.01$ for *in vitro* experiments and $P = 0.05$ for *in vivo* experiments.

3. Results

3.1. In vitro *antifungal activity*

Of the 38 aqueous leaf extracts tested at a final concentration of 20% (w/v), 29 had a significant ($P = 0.01$) antifungal activity against both *P. personata* and *P. arachidis* (Table I). Leaf extracts of *P. juliflora* and *L. esculentum* completely inhibited the germination of conidia of *P. personata* and urediniospores of *P. arachidis*. Additionally, leaf extracts of *Achras sapota* and *Cyamopsis tetragonolobus* against *P. personata*, and leaf extract of *Tagetus patula* against *P. arachidis* completely inhibited the spore germination. Except for *Citrus limon* and *Leucaena leucocephala* all the leaf extracts differed in their antifungal activity against both the test fungi and had a high antifungal activity against *P. arachidis* than *P. personata*. Leaf extracts of *P. betle* and *A. sapota* up to 5% (w/v), *C. tetragonolobus* up to 2% (w/v), and *P. juliflora* and *L. esculentum* up to 1% (w/v) concentration were significantly inhibitory to both *P. personata* and *P. arachidis* (Table II). In all the experiments percentage germination of conidia or urediniospores was >90% in control.

3.2. Greenhouse evaluation of leaf extracts

Leaf extracts of *P. juliflora* and *L. esculentum* were highly inhibitory to *P. arachidis* and *P. personata* up to

Table II. *In vitro* antifungal activity of different dilutions of selected aqueous leaf extracts against *Phaeoisariopsis personata* and *Puccinia arachidis*.

Plant species	Concentration (%, w/v)	Percentage spore germination	
		P. personata	*P. arachidis*
Achras sapota	5	76.3 (11)	11.8 (87)
	2	92.2 (−6)	64.7 (27)
	1	95.2 (−11)	89.6 (−3)
Cyamopsis tetragonolobus	5	7.1 (92)	28.6 (68)
	2	35.7 (59)	26.6 (70)
	1	73.1 (15)	55.3 (36)
Lycopersicon esculentum	5	0 (100)	0.5 (99)
	2	0 (100)	0 (100.0)
	1	1.3 (98)	0 (100.0)
Piper betle	5	59.0 (32)	53.9 (39)
	2	77.6 (10)	70.4 (21)
	1	67.9 (21)	74.0 (15)
Prosopis juliflora	5	0 (100)	0 (100)
	2	0.6 (99)	1.6 (98)
	1	1.6 (98)	2.1 (98)
Tagetus patula	5	83.6 (3)	58.6 (34)
	2	87.8 (−1)	76.9 (13)
	1	99.6 (−16)	97.4 (−12)
Control	5	86.2	88.6
	2	86.6	88.9
	1	85.6	86.9
SE_m (plant sp.)		0.77	0.76
SE_m (conc.)		0.51	0.50
SE_m (plant sp. × conc.)		1.34	1.32
F probability		<0.001	<0.001
Degrees of freedom		8	8

The values are the mean of nine replications in three sets of experiments. Values presented in parenthesis represent the percentage inhibition of spore germination in individual treatment with respect to control.

1% (w/v), and hence were evaluated for control of LLS and rust in greenhouse. Leaf extracts (2% w/v) applied as a prophylatic spray at different time intervals before the pathogen inoculation, had suppressive effects on the development of LLS and rust. In water-sprayed control plants LF of LLS ranged from 3.5 to 3.8, and was 7.9 to 8.4 for rust. The maximum reduction in LF of both LLS and rust was with simultaneous treatment of leaf extracts and inoculation. The increase in the time interval between the application of leaf extracts and pathogen inoculation corresponded with increase in LF of LLS and rust. Extract of *P. juliflora* gave a more durable protection against LLS and rust than that of *L. esculentum*, as observed by reductions in LF. Extract of *P. juliflora* applied at the same time as pathogen inoculation reduced LF of LLS and rust by 74.8 and 78.6%, compared to control. When the leaf extract was applied at 96 h before the pathogen inoculation this reduction was 35.7 and 50.7%. Leaf extract of *L. esculentum* was equally effective as *P. juliflora* extract in control of LLS and rust when applied 10 min before the pathogen. However, the reduction in LF of LLS and rust in *L. esculentum* treatment was only 5.7 and 12.5%, when it was applied at 96 h before the pathogen inoculation (Figure 1A,B).

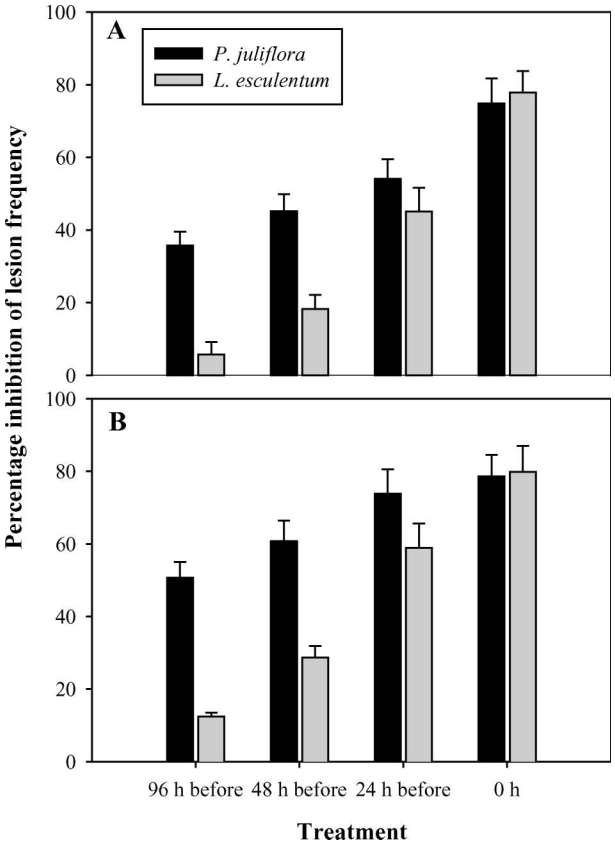

Figure 1. Greenhouse evaluation of aqueous leaf extracts of *Prosopis juliflora* and *Lycopersicon esculeutum* for control of (A) late leaf spot, and (B) rust diseases of groundnut. Aqueous leaf extracts (2%, w/v) were applied as a foliar spray at different time intervals before the pathogen inoculation and lesion frequency (number of lesions cm^{-2} leaf area) was measured 15 days after inoculation. The values are the mean of 12 replications in three sets of experiments.

3.3. Effect of P. juliflora *extract on the phenol metabolism of groundnut*

During LLS and rust infection, the total phenolic content of groundnut leaves (cv. TMV 2) increased up to three-fold compared to uninoculated control. Aqueous leaf extract of *P. juliflora* applied as a foliar spray did not significantly alter the phenol levels compared to pathogen control both during LLS and rust infections (Figure 2).

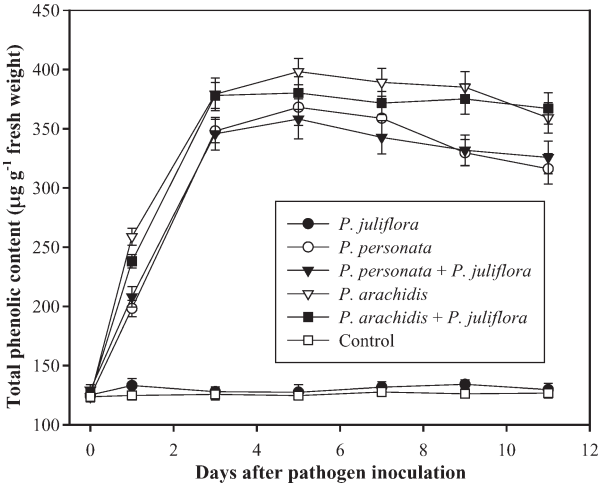

Figure 2. Effect of aqueous leaf extract of *Prosopis juliflora* on the phenolic content of groundnut leaves during *Phaeoisariopsis personata* and *Puccinia arachidis* infection. Leaf extract of *P. juliflora* (2%, w/v) was applied as a foliar spray on to 30-day-old plants of groundnut cv. TMV 2 at 24 h before the pathogen inoculation. Following pathogen inoculation, leaves were excised at different time intervals and the phenolic content was measured using Folin–Ciocalteau reagent. The values are the mean of nine replications in three sets of experiments.

3.4. Field evaluation of P. juliflora *leaf extract*

Leaf extract of *P. juliflora* (2% w/v) was tested both alone and in integration with chlorothalonil ($2\,g\,l^{-1}$) for control of combined infection of LLS and rust in field. In a repeated field experiment the disease development was greatest in control (disease score of 9.0 on a 1–9 rating scale) for definitive comparison with the treatment effects. The inhibitory effect of foliar application of *P. juliflora* leaf extract on the development of foliar diseases continued until harvest, and was highly significant ($P = 0.05$) up to 95 d.a.s. In three different treatments, leaf extract and chlorothalonil were applied in combination at different time intervals. Of these three treatments, the maximum disease control was obtained by the application of chlorothalonil at 60 d.a.s. and *P. juliflora* at 45, 75 and 90 d.a.s. In this treatment, the foliar diseases severity was rated 5.7 and 6.3 at harvest during 2002 and 2003 rainy seasons, compared to 9.0 in

control. Chlorothalonil applied for four times during the crop season remained the most effective among all the treatments (Figure 3A,B).

Similar to the disease severity, in both the years, highest pod yields were obtained by the repeated application of chlorothalonil from 45 to 90 d.a.s. (1.31 and 1.37 t ha^{-1}), followed by the application of chlorothalonil at 60 d.a.s. and *P. juliflora* at 45, 75 and 90 d.a.s. (1.21 and 1.19 t ha^{-1}) (Figure 4).

4. Discussion

There is a world-wide emerging trend to develop plant-based fungicides for use in different agricultural crops due to the increasing costs of synthetic fungicides, their accumulation in the food chain and adverse environmental effects. It is believed that natural fungicides will minimize the undesirable effects of synthetic fungicides and help preserve the environment for future generations in addition to reducing the input costs. It is in this context, we initiated exploring the fungicidal activity of commonly available plant spp. for control of LLS and rust.

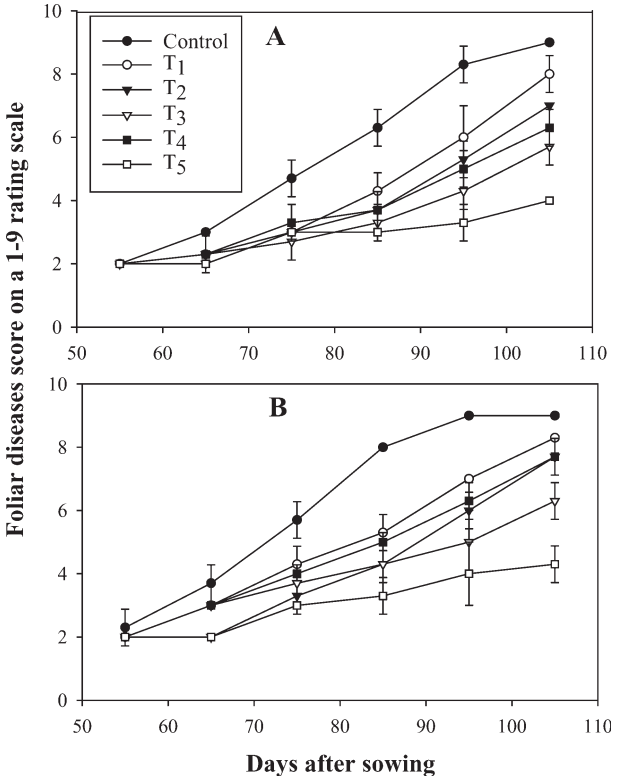

Figure 3. Field evaluation of aqueous leaf extract of *Prosopis juliflora* and/or chlorothalonil against combined infection of late leaf spot and rust diseases of groundnut during (A) year 2002 rainy season, and (B) year 2003 rainy season. T_1 = Leaf extract of *P. juliflora* (2%, w/v) at 45, 60, 75 and 90 d.a.s.; T_2 = *P. juliflora* extract at 60, 75 and 90 d.a.s., and chlorothalonil at 45 d.a.s.; T_3 = *P. juliflora* extract at 45, 75 and 90 d.a.s., and chlorothalonil at 60 d.a.s.; T_4 = *P. juliflora* extract at 45, 60 and 90 d.a.s., and chlorothalonil at 75 d.a.s.; T_5 = Chlorothalonil at 45, 60, 75 and 90 d.a.s.

In the preliminary *in vitro* studies, we observed different crude extracts to completely inhibit the germination of *P. personata* and *P. arachidis*. Similar potent antagonistic activity of plant extracts has been reported against *P. personata* and several other fungi (Kishore et al. 2001, Pinto et al. 1998). Extract of *P. juliflora* and *L. esculentum* remained highly antifungal even at 1% (w/v) concentration. These two plant spp. were well known for their antifungal activity. Extract of *P. juliflora* inhibited the germination of sclerotial *Rhizoctonia solani* (Ezhilan et al. 1994). The antifungal activity of *P. juliflora* extract could be largely due to the presence of alkaloids. Alkaloid fractions of *P. juliflora* extract were known for their antifungal activity (Ahmad et al. 1997). *L. esculentum* was known to possess the antifungal compounds rishitin and tomatine (Suleman et al. 1996). In presence of diluted leaf extracts of *A. sapota* and *T. patula*, there was an increase in the spore germination compared to control, probably because of the fungal utilization of available nutrients of these extracts.

The leaf extracts applied as a foliar spray were highly effective in control of LLS and rust in controlled environment. The efficacy of plant extracts decreased with an increase in the time interval between their application and pathogen inoculation. These results indicate the reduced persistence of plant extracts in the phylloplane. However, *P. juliflora* extract even at 96 h before the pathogen inoculation, significantly reduced the severity of LLS and rust. Extracts of *P. juliflora* and *L. esculentum* were observed to be more effective against LLS than extracts of *Azadirachta indica*, *D. metel* and *L. inermis*, earlier reported to be effective against LLS (Kishore

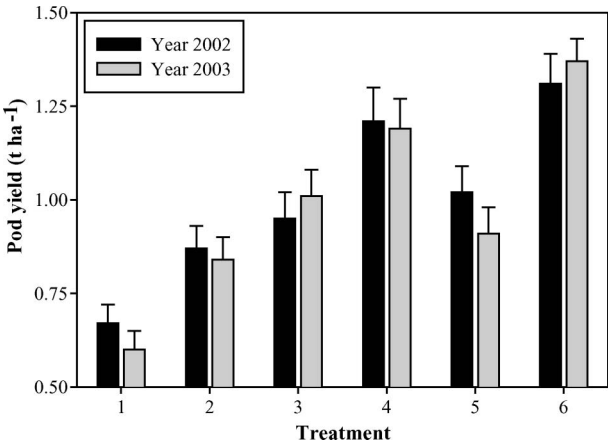

Figure 4. Effect of aqueous leaf extract of *Prosopis juliflora* applied both alone and in combination with chlorothalonil on the pod yield of groundnut during foliar diseases (LLS and rust) infection. Treatment 1 = Control; 2 = *P. juliflora* extract at 45, 60, 75 and 90 d.a.s.; 3 = *P. juliflora* extract at 60, 75 and 90 d.a.s., and chlorothalonil at 45 d.a.s.; 4 = *P. juliflora* extract at 45, 75 and 90 d.a.s., and chlorothalonil at 60 d.a.s.; 5 = *P. juliflora* extract at 45, 60 and 90 d.a.s., and chlorothalonil at 75 d.a.s.; 6 = chlorothalonil at 45, 60, 75 and 90 d.a.s.

et al. 2001). A few plant extracts have been shown to give control of foliar fungal diseases and often the disease control was equivalent to chemical fungicides. Extracts of *Reynoutria sachalinensis* protected the cucumber plants from powdery mildew infection by 90% and was comparable with that of fungicides myclobutanil and sulfur (Doltsinis and Schmitt 1998). Extract of *P. juliflora* was inhibitory to *Fusarium solani* and also reduced the Fusarium wilt in brinjal (Vimala et al. 1993).

Activation of host defense responses including cell wall thickening, and induction of phenolic compounds and PR-proteins has been demonstrated as one the mechanism involved in the protective action of plant extracts. Induction of phenolic compounds by extracts of *R. sachalenesis* imparted cucumber plants with resistance to powdery mildew infection (Daayf et al. 2000). The protective action of *P. juliflora* extract against blast of rice (Kamalakannan et al. 2001) and Alternaria blight of tomato (Thiribhuvanamala et al. 2001) is mediated by the enhanced activities of peroxidase, polyphenol oxidase and phenylalanine ammonia lyase in the host plants. In the present study, since *P. juliflora* had no effect on the host phenol metabolism against LLS and rust infections, it is assumed that the extract acts primarily as a contact fungicide.

The protective action of *P. juliflora* extract against the combined infection of LLS and rust was observed in field. However, the disease control was significant only up to 95 d.a.s. and a partial yield increase was obtained. Integrated use of *P. juliflora* extract and chlorothalonil improved the disease control. Optimized spray schedule for the combined application of *P. juliflora* extract and fungicide, indicated that one fungicide spray at 60 d.a.s. combined with three sprays of plant extract was effective in combined control of LLS and rust. This treatment increased the pod yield up to 81 – 98% compared to 96 – 129% obtained by repeated (4 times) application of chlorothalonil. Though the fungicide application at 45 d.a.s. was highly effective in foliar diseases control up to 75 d.a.s., further treatment with *P. juliflora* extract was not effective and there was a drastic increase in the disease severity. Fungicide application at 60 d.a.s., effected the secondary infection cycles resulting from the dispersal of fungal spores from initial lesions on bottom leaves and also infector rows. As a result, further treatments with *P. juliflora* extract continued to maintain the low disease severity. When the first two protective sprays were with plant extract, followed by chlorothalonil at 75 d.a.s., the fungicide had no drastic effect on the leaf area damage and defoliation resulting from the existing lesions.

The increase in pod yield obtained using *L. inermis* extract against LLS and rust was 15 – 40% (Ghewande 1989), and *D. metel* extract against LLS was 48% (Kishore et al. 2002). This study identified an effective combination of plant extract and fungicide to obtain maximum yields (81 – 98%) with minimal fungicide usage. Earlier attempts for integrated use of plant extracts and other disease management options were successful. Aqueous extracts of *A. indica* and *Glossocardia bosvallea* used in combination with carbendazim completely controlled the Sclerotium rot of potato. The disease control was higher than carbendazim and plant extracts used alone (Solunke et al. 2001). *P. juliflora* is a commonly available plant in the SAT and aqueous leaf extract is simple to prepare, hence integrated management of LLS and rust with *P. juliflora* extract and minimal use of fungicide may attain wider adoption through farmers' acceptance.

In view of the changing agricultural policies throughout the world, complete disease control is no longer a target of plant pathologists. Reducing the pest or pathogen populations below an economical threshold level using cost-effective and eco-friendly management option is the focus of the day. In this context, identification of aqueous leaf extract of *P. juliflora* as a fungicide effective against LLS and rust, is of highly significant.

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