CSIRO PUBLISHING

www.publish.csiro.au/journals/app *Australasian Plant Pathology*, 2005, **34**, 261–264

SHORT RESEARCH NOTES

Integrated applications of aqueous leaf extract of *Datura metel* and chlorothalonil improved control of late leaf spot and rust of groundnut

G. Krishna Kishore[A] *and S. Pande*[A,B]

[A]Pathology, International Crops Research Institute for the Semi-Arid Tropics, Patancheru 502 324, AP, India.
[B]Corresponding author. Email: s.pande@cgiar.org

Abstract. Late leaf spot (LLS) (causal agent *Phaeoisariopsis personata*) and rust (causal agent *Puccinia arachidis*) are economically important foliar diseases of groundnut (*Arachis hypogaea*) and have global significance. Aqueous leaf extracts of *Datura metel* and *Lawsonia inermis*, known for their high antifungal activity against *P. personata*, completely inhibited the germination of urediniospores of *P. arachidis in vitro*. In the greenhouse, extracts of *D. metel* (25 g/L) and *L. inermis* (50 g/L) applied as a prophylactic spray reduced the frequency of LLS lesions and rust pustules by 65–74% compared with controls. In field studies, a programme of four sprays of *D. metel* leaf extract at 45, 60, 75 and 90 days after sowing (DAS) was partially effective against the foliar diseases (LLS and rust) up to 95 DAS. A single spray of chlorothalonil at 45 DAS combined with three sprays of *D. metel* at 60, 75 and 90 DAS effectively reduced the combined severity of LLS and rust. The increase in pod yield by this treatment (91% over control) was comparable with the sustained application of chlorothalonil. Further validation of integrated applications of *D. metel* extract and chlorothalonil may reduce the dependency on fungicides in groundnut cultivation.

Additional keywords: antifungal, *Arachis hypogaea*, integrated disease management, natural fungicide, peanut.

Late leaf spot (LLS) (causal agent *Phaeoisariopsis personata*) and rust (causal agent *Puccinia arachidis*) are of global concern in groundnut (peanut) (*Arachis hypogaea*) production (Subrahmanyam *et al*. 1995). These two diseases usually occur in combination and can cause both haulm and pod yield losses of up to 70%. The need for repeated applications (3–4 sprays) of the recommended fungicides, such as carbendazim, chlorothalonil, mancozeb and tridemorph, discouraged extensive adoption of groundnut by resource-poor farmers of the rain-fed production systems. As a result, there is a need to identify alternative methods of disease management that are both economical and eco-friendly.

Crude extracts and purified secondary metabolites of several plant species inhibit the growth of diverse plant pathogenic fungi (Grayer and Harborne 1994). Fungicides sourced from plants potentially offer broad-spectrum activity, rapid degradation and low mammalian toxicity (Isman 2000) and may, therefore, be preferable to manufactured products. Plant-derived compounds or extracts were effectively used for control of blast of rice (*Oryza sativa*) caused by *Pyricularia oryzae* (Amadioha 2000), powdery mildew of cucumber (*Cucumis sativus*) caused by *Sphaerotheca fuliginea* (Daayf *et al*. 2000) and several other foliar diseases (Kishore and Pande 2004). Several preliminary investigations, which are reviewed by Podile and Kishore (2002), indicate the efficacy of plant extracts for the control of groundnut foliar diseases, including leaf spots and rust.

The objective of the present study was to identify potential plant-based fungicides for management of combined severity of LLS and rust of groundnut. Aqueous leaf extracts of *Datura metel* (downy thornapple, horn of plenty) and *Lawsonia inermis* (henna), earlier identified for high antifungal activity against *P. personata* (Kishore *et al*. 2001), were evaluated for antifungal activity against *P. arachidis in vitro* and control of LLS and rust in controlled environments. Further, integrated applications of leaf extracts and chlorothalonil were evaluated for improved disease control in the field.

Conidia of *P. personata* and urediniospores of *P. arachidis* were collected from diseased groundnut leaves. Single lesion and pustule isolates of *P. personata* and *P. arachidis* were maintained on groundnut cv. TMV 2 using a detached leaf technique (Subrahmanyam *et al*. 1995). Leaf extracts of

 10.1071/AP05025 0815-3191/05/020261

D. metel and *L. inermis* were prepared by homogenisation of fresh leaves in sterile distilled water followed by filtration and centrifugation of the filtrate (Kishore *et al*. 2001).

To determine the effect of plant extracts on urediniospore germination *in vitro*, 50 µL of urediniospore suspension (5×10^4 spores/mL) was mixed with an equal volume of aqueous leaf extracts of *D. metel* or *L. inermis* (final concentration of 0.4–200 g/L) on a cavity slide with sterile distilled water (SDW) as control. The slides were placed in a humid chamber and incubated at $25 \pm 1^\circ$C in the dark for 8 h and then observed for germination. One hundred urediniospores were observed in each treatment and the percentage inhibition of germination was calculated with respect to germination in the control (Kishore *et al*. 2001). There were three replications and the experiment was repeated once.

For greenhouse evaluation of plant extracts against LLS and rust, leaf extracts of *D. metel* and *L. inermis* were applied as a prophylactic spray, at concentrations where they completely inhibited the germination of *P. personata* and *P. arachidis in vitro*. The following treatments were applied as a foliar spray to 30-day-old greenhouse-grown groundnut plants of cv. TMV 2 (highly susceptible to LLS and rust): (i) leaf extract of *D. metel* (25 g/L), (ii) leaf extract of *L. inermis* (50 g/L), (iii) chlorothalonil (Kavach) (2 g/L), and (iv) SDW as control. After 24 h, the plants were challenge-inoculated with *P. personata* or *P. arachidis* inoculum (2×10^4 conidia or urediniospores/mL). Inoculated plants were incubated at $24 \pm 2^\circ$C alternately in dew chambers (Clifford 1973) for 16 h and in a greenhouse for 8 h, to maintain alternate wet and dry periods up to 8 days after inoculation (DAI). Severity of LLS and rust was measured as (a) lesion or pustule frequency (number of lesions or pustules/cm^2 leaf area) at 15 DAI, (b) percentage necrotic area (PNA) at 30 DAI and (c) disease score on a 1–9 rating scale (1 = no disease and 9 = maximum disease) at 30 DAI. Lesion or pustule frequency and PNA were measured on the leaf that was fourth from the top of each plant at the time of inoculation. Each treatment consisted of three replicates of 12 plants and the whole experiment was done three times.

Field evaluation of plant extracts for simultaneous control of LLS and rust was conducted at the International Crops Research Institute for the Semi-Arid Tropics, Patancheru, India during the 2001 and 2002 rainy seasons (June–October). The experiments were arranged in a completely randomised block design with three replications of each treatment. In each experimental plot consisting of four rows of 9 m length, groundnut cv. TMV 2 was planted with an intra- and inter-row spacing of 15 and 60 cm, respectively. One infector-cum-indicator row of TMV 2 was planted on either side of each plot and different treatments were separated by buffers of two rows of soybean. At 35 days after sowing (DAS), the infector-cum-indicator rows were spread with LLS- and rust-infected crop debris from the previous season and further inoculated with an equal mixture of conidia and urediniospores suspension (10^4/mL) of *P. personata* and *P. arachidis*. Following inoculation, sprinkler irrigation was provided from 1800 to 1830 h on all rain-free days to maintain leaf wetness, until 90 DAS. The following treatments were applied as a foliar spray to determine their effect on the combined severity of LLS and rust: (i) *D. metel* extract (25 g/L) at 45, 60, 75 and 90 DAS, (ii) *L. inermis* extract (50 g/L) at 45, 60, 75 and 90 DAS, (iii) chlorothalonil (2 g/L) at 45 DAS and *D. metel* extract at 60, 75 and 90 DAS, (iv) chlorothalonil at 45 DAS and *L. inermis* extract at 60, 75 and 90 DAS, (v) chlorothalonil at 45, 60, 75 and 90 DAS, and (vi) SDW at 45, 60, 75 and 90 DAS as control. Combined severity of LLS and rust was scored on a 1–9 rating scale (Subrahmanyam *et al*. 1995) at 10-day intervals from 45 DAS till 105 DAS. At harvest (110 DAS), the hand-picked pods from the uprooted plants were sun-dried and the recorded haulm and pod yields were calculated as tonnes/ha. The disease severity, haulm and pod yields were similar in the two crop seasons and hence the data were pooled and subjected to analysis of variance (ANOVA) using the Genstat 5 statistical package.

Aqueous leaf extracts of *D. metel* at 25 g/L and *L. inermis* at 50 g/L completely inhibited the germination of urediniospores of *P. arachidis* (Table 1). *D. metel* extract at lower concentrations was more inhibitory to urediniospore germination than was *L. inermis* extract of similar concentration (Table 1). These two extracts were known to completely inhibit the conidial germination of *P. personata in vitro* (Kishore *et al*. 2001). Leaf extract of *D. metel* also completely inhibited the conidial germination of *Colletotrichum capsici in vitro* (Gomathi and Kannabiran 2000) and its antifungal activity was heat stable and unaltered after storage for 180 days at room temperature (~28°C) (Kishore *et al*. 2001).

Table 1. The effect of various concentrations of aqueous leaf extracts of *Datura metel* and *Lawsonia inermis* on urediniospore germination of *Phaeoisariopsis arachidis in vitro*

Concentration (g/L)	Percentage inhibition of urediniospore germination[A]	
	D. metel	*L. inermis*
200.0	100.0	100.0
100.0	100.0	100.0
50.0	100.0	100.0
25.0	100.0	88.9
12.5	95.9	78.6
6.3	88.7	61.8
3.1	79.8	37.3
1.6	63.2	12.8
0.8	51.9	2.5
0.4	43.8	0.0

[A]Percentage inhibition of urediniospore germination relative to germination in control. The values are the means of six replicates from two repeats of the experiment.

Table 2. Severities of late leaf spot (LLS) and rust following prophylactic application of leaf extracts of *Datura metel* or *Lawsonia inermis* and chlorothalonil in a greenhouse experiment

Treatment	Lesion/pustule frequency[A]		Percentage necrotic area		Disease score on a 1–9 rating scale	
	LLS	Rust	LLS	Rust	LLS	Rust
D. metel (25 g/L)	1.39	2.18	16.2	19.8	3.5	3.4
L. inermis (50 g/L)	1.68	2.56	20.5	24.2	4.0	4.1
Chlorothalonil (2 g/L)	0.16	0.28	4.0	3.0	2.0	2.0
Control	4.49	8.51	-[B]	100	9.0	9.0
LSD ($P = 0.01$)	0.62	0.83	1.3	15.8	0.6	0.6

[A]Lesion or pustule frequency was measured 15 days after inoculation (DAI), and percentage necrotic area and disease score were recorded 30 DAI. The values are the mean of nine replicates from three repeats of the experiment.
[B]100% defoliation was observed.

In the greenhouse, prophylactic application of leaf extracts of *D. metel* (25 g/L) and *L. inermis* (50 g/L) reduced LLS lesion frequency by 69% and 63%, and rust pustule frequency by 74% and 70%, respectively, compared with the control. Reduced severity of LLS and rust in these treatments was also evident from other components of disease scoring i.e. PNA and disease score on a 1–9 rating scale (Table 2). The protective effect of plant extracts is supported by earlier findings where plant extracts were effective contact fungicides and/or activators of host plant resistance (Daayf *et al*. 2000) that are often comparable with synthetic fungicides (Amadioha 2000). Leaf extract of *D. metel* induced peroxidase and polyphenol oxidase activities in *Capsicum annuum* (Asha and Kannabiran 2001) and may also induce the same in groundnut. The inhibitory effect of *D. metel* and *L. inermis* extracts on the severity of groundnut LLS and rust can be compared with earlier findings that plant extracts including neem (*Azadirachta indica*) oil, neem seed kernel extract (reviewed by Podile and Kishore 2002), limonoids from *A. indica* (Suresh *et al*. 1997), and a tetranotriterpenoid compound from *Toona ciliata* (Govindachari *et al*. 2000) were effective in management of groundnut LLS or rust or both.

The two foliar diseases LLS and rust usually occur in combination, hence the leaf extracts were evaluated for simultaneous control of LLS and rust in the field. Leaf extract of *L. inermis* applied alone or in integration with chlorothalonil and extract of *D. metel* significantly ($P = 0.01$) reduced the severity of the foliar diseases up to 95 DAS. Integrated applications of *D. metel* extract and chlorothalonil were more effective than *D. metel* extract alone, and comparable with chlorothalonil until 95 DAS. At 105 DAS, the foliar diseases severity in this integrated treatment was 5.7 compared with 4.0 in the chlorothalonil treatment (Fig. 1). Integrated applications of plant extracts and fungicides or biocontrol agents have been successful in improved control of anthracnose and pod blight of soybean (Chandrasekaran and Rajappan 2002) and Sclerotium rot of potato (Solunke *et al*. 2001).

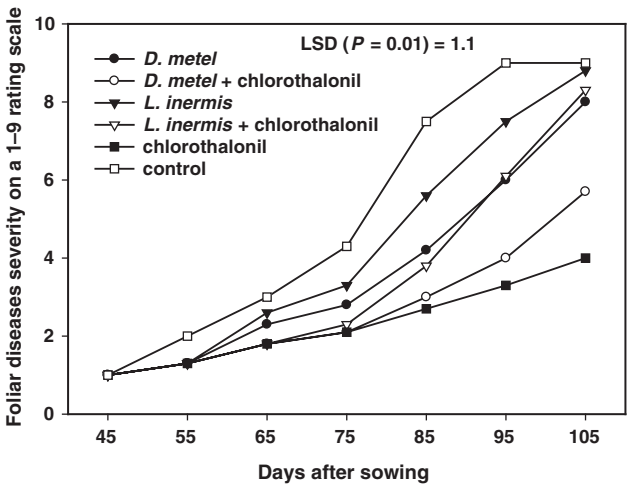

Fig. 1. Effect of integrated applications of aqueous leaf extracts of *Datura metel* or *Lawsonia inermis* and chlorothalonil on the combined severity of late leaf spot and rust diseases of groundnut in field. Leaf extracts of *D. metel* (25 g/L), *L. inermis* (50 g/L), and chlorothalonil (2 g/L) were sprayed at 45, 60, 75 and 90 DAS. In integrated applications of leaf extracts and chlorothalonil, the fungicide was applied at 45 DAS followed by *D. metel* or *L. inermis* leaf extract at 60, 75 and 90 DAS. Combined infection of late leaf spot (LLS) and rust was measured on a 1–9 rating scale (Subrahmanyam *et al*. 1995). Data points are the mean of six replicates of a repeated field experiment.

Foliar application of *D. metel* extract by itself increased the haulm and pod yields by 13 and 52%, respectively, whereas the increase was 33 and 91% following integrated applications of *D. metel* extract and chlorothalonil (Table 3). The pod yields in the latter treatment were comparable with those following repeated applications of chlorothalonil, since the foliar diseases severity was the same during pod formation and filling stages. Leaf extract of *L. inermis*, both alone and in integration with chlorothalonil, had no significant effect on haulm yields, but increased the pod yields by 17% and 20%, respectively (Table 3). The partial increase of pod yields in leaf extract treatments was similar to that found by Ghewande (1989), who identified the efficacy of *L. inermis*

Table 3. Effect of integrated applications of aqueous leaf extracts of *Datura metel* or *Lawsonia inermis* and chlorothalonil on the haulm and pod yields of groundnut during combined infection of late leaf spot and rust diseases in the field

Treatment	Yield (t/ha)	
	Haulm	Pod
D. metel (25 g/L)[A]	1.07	0.82
L. inermis (50 g/L)[A]	0.95	0.63
D. metel (25 g/L) and chlorothalonil (2 g/L)[B]	1.26	1.03
L. inermis (50 g/L) and chlorothalonil (2 g/L)[B]	0.96	0.65
Chlorothalonil (2 g/L)[A]	1.43	1.12
Control	0.95	0.54
LSD ($P = 0.01$)	0.13	0.10

[A]All treatments were applied as a foliar spray at 45, 60, 75 and 90 days after sowing (DAS).
[B]Chlorothalonil was sprayed at 45 DAS and leaf extracts at 60, 75 and 90 DAS.
All values are the mean of six replicates from two repeats of the experiment.

extract in control of groundnut foliar diseases resulting in an increase of 15–40% in pod yield. Extracts of *D. metel* were known for their potent broad-spectrum antifungal activity and the plant is commonly available in the semi-arid tropics. Integrated applications of chlorothalonil and *D. metel* extract reduced the dependency on chemical fungicides to one quarter of that normally used for management of groundnut foliar diseases. Further large-scale on-farm validation of integrated applications of *D. metel* extract and chlorothalonil is under progress for wider adoption of this eco-friendly and economic disease control strategy by groundnut-growing farmers.

The Department for International Development (DFID), UK supported this work under the research project R7346.

References

Amadioha AC (2000) Controlling rice blast *in vitro* and *in vivo* with extracts of *Azadirachta indica*. *Crop Protection* **19**, 287–290. doi: 10.1016/S0261-2194(99)00080-0

Asha AN, Kannabiran B (2001) Effect of *Datura metel* leaf extract on the enzymatic and nucleic acid changes in the chilli seedlings infected with *Colletotrichum capsici*. *Indian Phytopathology* **54**, 373–375.

Chandrasekaran A, Rajappan K (2002) Effect of plant extracts, antagonists and chemicals (individual and combined) on foliar anthracnose and pod blight of soybean. *Journal of Mycology and Plant Pathology* **32**, 25–27.

Clifford BC (1973) The construction and operation of a dew simulation chamber. *The New Phytologist* **77**, 619–623.

Daayf F, Ongena M, Boulanger R, El-Hadrami I, Belanger RR (2000) Induction of phenolic compounds in two cultivars of cucumber by treatment of healthy and powdery mildew infected plants with extracts of *Reynoutria sachalenensis*. *Journal of Chemical Ecology* **26**, 1579–1593. doi: 10.1023/A:1005578510954

Ghewande MP (1989) Management of foliar diseases of groundnut (*Arachis hypogaea*) using plant extracts. *Indian Journal of Agricultural Sciences* **59**, 133–134.

Gomathi V, Kannabiran B (2000) Inhibitory effects of leaf extracts of some plants on the anthracnose fungi infecting *Capsicum annuum*. *Indian Phytopathology* **53**, 305–308.

Govindachari TR, Suresh G, Gopalakrishnan G, Masilamani S, Banumathi B (2000) Antifungal activity of some tetranortriterpenoids. *Fitoterapia* **71**, 317–320. doi: 10.1016/S0367-326X(99)00155-0

Grayer RJ, Harborne JB (1994) A survey of antifungal compounds from higher plants, 1982–1993. *Phytochemistry* **37**, 19–42. doi: 10.1016/0031-9422(94)85005-4

Isman MB (2000) Plant essential oils for pest and disease management. *Crop Protection (Guildford, Surrey)* **19**, 603–608. doi: 10.1016/S0261-2194(00)00079-X

Kishore GK, Pande S (2004) Natural fungicides for management of phytopathogenic fungi. *Annual Review of Plant Pathology* **3**, (In press).

Kishore GK, Pande S, Rao JN (2001) Control of late leaf spot of groundnut (*Arachis hypogaea*) by extracts from non-host plant species. *The Plant Pathology Journal* **17**, 264–270.

Podile AR, Kishore GK (2002) Biological control of peanut diseases. In 'Biological control of crop diseases'. (Ed. SS Gnanamanickam) pp. 131–160. (Marcel Dekker Inc.: New York)

Solunke BS, Kareppa BM, Gangawane LV (2001) Integrated management of Sclerotium rot of potato using carbendazim and plant extracts. *Indian Journal of Plant Protection* **29**, 142–143.

Subrahmanyam P, Mc Donald D, Waliyar F, Reddy LJ, Nigam SN, *et al*. (1995) Screening methods and sources of resistance to rust and late leaf spot of groundnut. Information Bulletin No. 47. *Patancheru* 502, 324 . [Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics]

Suresh G, Narasimhan NS, Masilamani S, Partho PD, Gopalakrishnan G (1997) Antifungal fractions and compounds from uncrushed green leaves of *Azadirachta indica*. *Phytoparasitica* **25**, 33–39.

Received 6 August 2004, accepted 16 December 2004