

Potential Use of Nettle (*Urtica dioica* L.) Extracts for Management of Alternaria Blight of Radish

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ABSTRACT. Laboratory and field experiments were conducted to investigate the effectiveness of nettle (*Urtica dioica* L.) extracts for the management of alternaria blight of radish incited by *Alternaria* spp. at Rampur, Nepal, during 2001/2002. Effectiveness tests for inhibition of spore germination and mycelial growth were carried out in the laboratory on glass slides and poisoned media, respectively. A field experiment was conducted in a split-plot design with spray frequencies as the main-plot and different concentrations of fresh and fermented nettle extracts and mancozeb as sub-plot factors.

Mancozeb appeared to be superior to all other treatments in suppressing conidia germination and mycelial growth in laboratory experiments and disease in the field. Among nettle extracts, fresh nettle extracts in cattle urine was found to be the best under laboratory and field conditions, followed by fresh nettle extracts in water and fermented nettle extract. Fresh nettle extract in cattle urine, diluted to 5% and 10% in water, showed second highest level of suppression of conidia germination and fungal growth, respectively, after mancozeb. Fresh nettle sap extracted in cattle urine at 20% dilution level showed the highest level of disease suppression in field and also produced the highest radish yield among nettle extract treatments. Use of fresh nettle extracts in cattle urine seems to be a potential measure for the management of alternaria blight of radish.

INTRODUCTION

Blight symptoms caused by *Alternaria* spp. is considered as the main disease of radish (*Raphanus sativus* L.) affecting both, fresh vegetable and seed production. In Nepal, yield reduction up to 45% in radish has been reported from Kathmandu and Chitwan valley, due to alternaria blight (Shrestha, 1996). Similar results have also been reported on other *Brassica* species such as rapeseed and mustard (Kolte and Tiwari, 1989).

Different chemicals, either as a seed treatment or foliar spray, have been proved effective in controlling alternaria blight (Kolte and Tiwari, 1989). However, intensive and indiscriminate use of those chemicals may also have adverse effects on crops, antagonistic organisms, and environment as well as human and animal health. Besides, the most serious problem might be the development of fungicide-resistant strains of the fungus leading to a failure in disease control (De Waard *et al.*, 1993).

In this regard, recent research has been directed towards the development of ecofriendly measures of disease control, such as biological control, induced resistance (Lyon *et al.*, 1995) and use of biodegradable natural products, especially from medicinal plants (Kaitisha, 1995). Several researchers have reported antifungal properties of crude plant extracts inhibiting different species of *Alternaria* (Muthulakshmi and Seetharaman, 1993; Senthilnathan and Nagarasimhan, 1993; Tytkowaska and Dorna, 2001).

Nettle (*Urtica dioica* L.) of the family Urticaceae, is a perennial, wild plant growing worldwide in wastelands. In Nepal, it has been considered a plant species with antifungal and insecticidal properties. Plant parts and fruit extracts of nettle and timur (*Zanthoxylum* spp.) have been found effective in controlling different insects, such as cabbage butterfly (*Pieris brassicae nepalensis* Doubleday), hairy caterpillar (*Spilarctia casignata* Kollar), beetle (*Monolepta signata* Oliver), stem borer (*Earias* spp), and different species of aphids, thrips, and diseases, such as bean rust (*Uromyces*

appendiculatus) and leaf curl virus (Budhathoki, 1992). Young nettle leaves and stems steeped in water for 7 days and the suspension diluted 4 times in water was found effective when sprayed against caterpillars, aphids and ants of fruits, vegetables and pulses, and disease such as powdery mildew of pea (Regmi and Kerna, 1988). Authors are aware that traditionally the farmers in Myagdi district, Nepal use nettle extract to manage different diseases and insects of crop plants.

However, research based information on the effectiveness of nettle extracts in controlling alternaria blight of radish is lacking. Therefore, the study was carried out to investigate the effectiveness of nettle extracts in inhibiting spore germination and mycelial growth of *Alternaria* spp. *in-vitro* to control alternaria blight of radish in the field.

MATERIALS AND METHODS

Preparation of nettle extracts

Nettle extract was obtained by three different methods, viz. (i) by fermenting chopped nettle leaves and stems in water, (ii) by grinding fresh nettle leaves and stems in water, and (iii) by grinding fresh nettle leaves and stems in cattle urine. For fermentation, five kg of chopped, fresh nettle leaves and stems was immersed in five liters of water were kept in a plastic bucket and kept airtight for 15 days. On the 15th day, nettle extract was obtained by squeezing the contents and straining the mixture firstly with a mosquito net and then using a double layer of muslin cloth. The extract was considered as 100% stock solution and used immediately after dilution with water as per the requirement (Muthulakshmi and Seetharaman, 1993). For fresh extractions, five kilograms of nettle leaves and stems was ground by using a mortar and pestle with five liters of water. Similarly five kilograms of nettle leaves and stem was ground with five liters of cattle urine. The suspensions were squeezed-strained as above. These extracts were also considered as 100% stock solutions and used immediately after dilution with water.

Effectiveness test of nettle extracts and mancozeb *in-vitro*

Inhibition of spore germination

Nettle extracts and mancozeb (Dithane[®] M-45; 75% WP) were tested for their effectiveness in inhibition of conidia (spore) germination of *Alternaria* spp. isolated from naturally infected radish plants *in-vitro*. There were 13 treatments (Table 1) with four replications.

One drop of spore suspension was mixed with one drop of each treatment solution on separate cavity slides. The slides were placed in petri plates containing three layers of moistened filter paper and incubated at 25±2°C. After 48 h, germinated conidia of each slide and total conidia were counted from ten randomly selected, microscopic fields and germination percentage was calculated.

Inhibition of mycelial growth

For the studies on suppression of mycelial growth of *Alternaria* spp., the same 13 treatments as described in Table 1 were tested by poisoned food technique (Muthulakshmi and Seetharaman, 1993) with five replications. The suspensions of the 13 treatments were added to separate potato dextrose agar (PDA) media (PDA powder 39 g and distilled water 1000 ml) just prior to pouring, when temperature was about 45-50°C, in such quantities that the final concentration of each treatment resulted as given in Table 1. Streptomycin sulphate was also added to all PDA media to a final concentration of 200 ppm to avoid bacterial contamination. The media were stirred and poured about 20 ml/petri plate. The

cooled media were inoculated with a pure culture of *Alternaria* species of a five day old culture by placing 5 mm discs, one at the center of each petri plate. The plates were incubated at $25\pm 2^{\circ}\text{C}$ and the radial growth of fungus was recorded at 8th day after incubation.

Table 1. Treatments used for inhibition of spore germination and mycelial growth of *Alternaria* spp. *in-vitro*, at Rampur, 2001/2002.

Treatment number	Treatment	Concentrations used against	
		spore germination	mycelial growth
1	Dithane M-45 (Mancozeb 75 WP)	100 ppm	50 ppm
2	Dithane M-45 (Mancozeb 75 WP)	200 ppm	100 ppm
3	Dithane M-45 (Mancozeb 75 WP)	400 ppm	200 ppm
4	Fresh nettle extract in water	2.5%	1.25%
5	Fresh nettle extract in water	5%	2.5%
6	Fresh nettle extract in water	10%	5%
7	Fresh nettle extract in cattle urine	2.5%	1.25%
8	Fresh nettle extract in cattle urine	5%	2.5%
9	Fresh nettle extract in cattle urine	10%	5%
10	Fermented nettle extract in water (15 days)	2.5%	1.25%
11	Fermented nettle extract in water (15 days)	5%	2.5%
12	Fermented nettle extract in water (15 days)	10%	5%
13	Control (distilled water)	-	-

Percentage inhibition of spore germination and mycelial growth was calculated as described by Muthulakshmi and Seetharaman (1993), using the equation (1);

$$I = \frac{(C - T)}{C} \times 100 \quad (1)$$

where,

I = Inhibition of spore germination or mycelial growth (%)
 C = Spore germination (%) or mycelial growth (cm) in control
 T = Spore germination (%) or mycelial growth (cm) in treatment

Field experiments with nettle extract and mancozeb

Layout of field and production of radish plants

The experiment was conducted at Rampur, Chitwan, Nepal during 2001-2002. The field was laid out in a split-plot design. Three main plots and seven subplots were replicated thrice. Sub-plot size was 2.25×1.5 m, and 50 cm space between two sub-plots. Space between two main plots and replications was 1 m. Radish cultivar "Forty Days" was sown on 18th October 2001 in a well-prepared soil, at a spacing of 45×30 cm, and three

seeds per hill. One plant per hill was maintained 20 days after sowing. The field was fertilized using diammonium phosphate, urea and muriate of potash at 40 P, 100 N and 40 K kg/ha. Seventy-five kg N and full dose of P and K were applied at the time of final land preparation and 25 kg N was top dressed 35 days after sowing (DAS). Other cultural operations were done as required.

Application of treatments

Main plot treatments were two, four and six sprays given to radish plants. Two sprays were given 95 and 115 DAS; four sprays 85, 100, 115 and 130 DAS and six sprays 85, 95, 105, 115, 125 and 135 DAS. There were seven subplot treatments. Stock solutions (100%) of nettle extracts were diluted to 10 or 20% with water, and the treatments were: fresh nettle extract in water (10 and 20%), fresh nettle extract in cattle urine (20%), nettle extract fermented in water for 15 days (10 and 20%), mancozeb (Dithane[®] M-45; 75% WP) (0.2%) and water as the control.

Assessment of disease

Disease observations were taken from five mature green leaves, one each of five interior plants selected randomly. The leaves were tagged for future scoring. Disease scoring was started 82 days after sowing, prior to application of the treatments. Other observations were taken 97, 107 and 117 days after sowing after applying the treatments. Disease intensity was scored in a 0-5 scale as given below:

- 0 = No symptoms,
- 1 = 1-10% leaf area covered by spots
- 2 = 11-25% leaf area covered by spots
- 3 = 26-50% leaf area covered by spots
- 4 = 51-75% leaf area covered by spots, and
- 5 = > 75% leaf area covered by spots

Percentage disease index (PDI) was calculated using the formula (equation 2) described by Sharma and Kolte (1994).

$$PDI = \frac{\text{Total of all numerical ratings}}{\text{Total number of leaves observed} \times \text{Maximum category value (5)}} \times 100 \quad (2)$$

Number of spots per 25 cm² leaf area was counted and size of 15 spots/plot was measured 82 and 97 days after sowing. Number of spots on 10 cm long raceme, just below the last branch of central inflorescence axis, and number of spots on 10 siliques, developed on main raceme, were counted from the five tagged plants and mean was calculated. Overall rating of disease on the whole inflorescence was done from nine interior plants, 147 days after sowing, using a 0-5 scale as follows:

- 0 = No lesion (healthy inflorescence)
- 1 = Infection on leaf and started on stem
- 2 = 5% area of stem covered by lesions
- 3 = 6-25% area of stem covered by lesions, initiation of lesion on siliques,
- 4 = 50% area of stem covered by lesions and sufficient lesions on siliques,
- 5 = >50% area of inflorescence and siliques covered by lesions.

Per cent disease index (PDI) was calculated using equation (2).

Seed yield

Number of siliques per plant was counted from five tagged plants 170 DAS. After harvesting, seed yield per plot and 1000 seed weight was taken at 8% moisture level.

Determination of seed infection by *Alternaria* spp.

One hundred, randomly selected seeds from each treatment, were placed on water agar medium in petri plates. The plates were incubated at $25\pm 2^\circ\text{C}$ for three days. Seeds showing spores of *Alternaria* species were counted under a stereomicroscope and the percentage of seed infection was calculated.

Statistical analysis

Count data were transformed into log values and percentage data were transformed into arc sine values (Gomez and Gomez, 1984). Data were analyzed by analysis of variance. Means were compared by Duncan's Multiple Range Test. Statistical software used for data analysis were Microsoft Excel-2000 and MSTATC-1990 microcomputer programs.

RESULTS AND DISCUSSION

Inhibition of spore germination and mycelial growth

All treatments except 1.25% fermented nettle extract in water, inhibited spore germination and mycelial growth significantly as compared to the control. Mancozeb inhibited spore germination completely whereas mycelial growth was suppressed only up to 60% at 400 ppm over control (Table 2). The three treatments of Mancozeb differed significantly from other treatments, but not within themselves, for inhibiting spore germination, while only 400 ppm significantly reduced (2.18 cm) mycelial growth as compared to all the other treatments. Effect of 100 ppm mancozeb on mycelial growth did not vary considerably from that of 5 and 10% fresh nettle extract in cattle urine, 10% fresh nettle extract in water and 10% fermented nettle extract (Table 2). In general, fresh nettle extracts in cattle urine showed the highest level of inhibition on spore germination and mycelial growth when compared to fresh and fermented extracts in water. Fresh nettle extract in cattle urine diluted to 10% in water caused a significantly higher (93%) inhibition of conidia germination compared to all the other nettle extracts.

The results indicated that fresh nettle extracts consisted of certain chemicals, which inhibits spore germination and fungistatic to mycelial growth of *Alternaria* species of radish. Cattle urine appeared to be synergistic with fresh nettle extract for diminishing germination and growth of the pathogen. Fermented nettle extract showed a low antifungal activity compared to the fresh nettle extract.

These findings are in agreement with the results reported by Senthilnathan and Narasimhan (1993) disease management using plant extracts where 10% leaf extract of *Aegle marmelos* and *Prosopis juliflora* were effective in inhibiting spore germination and mycelial growth of *Alternaria tenuissima*, the causal fungus of leaf blight of onion, and mycelial growth of *Alternaria tenuis* inciting fruit rot of chilli *in-vitro* (Muthulakshim and Seetharaman, 1993). Similarly, ginger (*Zingiber officinale*) extracts effectively inhibited the mycelial growth of *A. brassicae* and *A. brassicicola* (Tylkowaska and Dorna, 2001).

Table 2. Inhibition of conidia germination and mycelial growth of *Alternaria* spp. isolated from radish, by mancozeb and nettle extracts *in-vitro*.

Treatments for		Conidia germination (%)	Inhibition of conidia germination (%)	Mycelial growth (cm)	Inhibition of mycelial growth (%)
Conidia germination	Mycelial growth				
Mancozeb					
50 ppm	100 ppm	0.00 (0.25) ¹ h	100 (99.62)	3.26 de	41
100 ppm	200 ppm	0.00 (0.25) h	100 (99.62)	2.82 e	49
200 ppm	400 ppm	0.00 (0.25) h	100 (99.62)	2.18 f	60
Fresh nettle extract in water					
1.25%	2.5%	68.80 (56.09) c	19 (17)	4.48 b	18
2.5%	5%	44.13 (41.62) d	48 (39)	4.50 b	18
5%	10%	18.13 (26.51) e	79 (61)	3.52 d	36
Fermented nettle extract in water					
1.25%	2.5%	0.63 (63.97) ab	6 (6)	5.04 a	8
2.5%	5%	4.88 (60.17) bc	12 (11)	4.56 b	17
5%	10%	37.90 (37.94) d	56 (44)	3.62 cd	34
Fresh nettle extract in cattle urine					
1.25%	2.5%	17.66 (24.39) ef	79 (64)	4.08 bc	26
2.5%	5%	11.65 (19.87) f	86 (71)	3.70 cd	32
5%	10%	6.375 (14.40) g	93 (79)	3.38 d	38
Control (Water)	-	85.45 (67.73) a	-	5.48 a	-
LSD (p = 0.05)		7.073 (4.974)	-	0.448	-
CV%		10.93		9.08	

¹ Figures in the parenthesis are arc sine values. Means within a column followed by the same letter do not differ significantly by DMRT at P > 0.05.

Suppression of alternaria blight on vegetative parts of radish

Sixty days after sowing, leaf spots started to appear on older leaves. Development of disease was the lowest (22.67-44.00%) in mancozeb sprayed plots than all the nettle extracts sprayed (38.67-87.56%) and control (50.67-91.56%) plots in all observation dates (Table 3). After mancozeb, fresh nettle sap extracted in cattle urine was found to be the best followed by fresh nettle sap extracted in water, to suppress the disease. Disease index percentage of leaves of the plots sprayed with 20% fresh nettle extract in cattle urine (78%) was significantly higher than in all the other nettle extract sprayed plots but at par with

plots sprayed with 20% fresh nettle extract in water, 117 DAS (Table 3). Number and size of leaf spots were also the lowest in the plots sprayed with fresh nettle extract in water and fresh nettle extract in cattle urine at 20% concentration when compared to the plots sprayed with other nettle treatments (data not shown). Fresh extracts as well as 20% concentration of extracts appeared more effective than fermented extracts as well as the lower concentration (10%).

Table 3. Effect of nettle extracts and mancozeb on alternaria blight disease index on leaf and inflorescence of radish, at Rampur, Nepal 2001-2002.

Treatments	Disease index leaves (%)			Disease index inflorescence (147 DAS) %
	97 DAS ¹	107 DAS	117 DAS	
Fresh nettle extract in water (20%)	40.89 b	60.89 c	80.22 cd	62.25 d
Fresh nettle extract in water (10%)	41.11 b	75.56 b	87.11 b	69.60 c
Fermented nettle extract in water (20%)	43.11 b	62.22 c	81.78 c	66.65 cd
Fermented nettle extract in water (10%)	43.11 b	77.78 b	87.56 b	78.26 b
Fresh nettle extract in cattle urine (20%)	38.67 b	61.33 c	78.00 d	63.28 d
Mancozeb (0.2%)	22.67 c	37.78 d	44.00 e	30.41 e
Control	50.67 a	84.44 a	91.56 a	86.80 a
CV%	15.66	7.99	3.06	6.95

Means within each column followed by same letter do not differ significantly by DMRT at P>0.05. ¹Days after sowing.

Suppression of alternaria blight on reproductive parts of radish

Disease development on inflorescence was the lowest (30.4%) on mancozeb sprayed plots and highest (86.8%) on control plots 147 DAS (Table 3). Among nettle extracts, fresh nettle extract in water gave the lowest disease incidence, followed by fresh nettle extract in cattle urine at 20% concentration. Number of disease spots on siliques were also the lowest on mancozeb sprayed plots followed by plots sprayed with fresh nettle extract in cattle urine (data not shown).

Seed yield

Seed yield and 1000 seed weight were the highest in mancozeb treated plots (859.1 kg/ha and 12.79 g, respectively), followed by plots treated with fresh nettle extract in water and fresh nettle extract in cattle urine for seed yield, and fresh nettle extract in cattle urine and fermented nettle extract in water for 1000 seed weight (Table 4). However, there was no considerable difference in seed yield among plots sprayed with different nettle extracts, and in 1000 seed weight between plots sprayed with nettle extract in cattle urine and fermented nettle extract in water at 20% concentration. Both, seed yield and 1000 seed weight were the lowest from control plots. Higher seed yields were attributed to lower disease intensity, higher silique number per plant and bolder seeds.

Table 4. Effect of nettle extracts and mancozeb on seed yield and percentage seed infection of radish plants diseased with alternaria blight, in field experiments (2001-2002).

Treatment	Seed yield (kg/ha)	Thousand seed weight (g)	Seed infection (%)
Fresh nettle extract in water (20%)	623.5 b	11.74 cd	77.78 b
Fresh nettle extract in water (10%)	576.8 b	11.40 de	79.11 ab
Fermented nettle extract in water (20%)	589.6 b	11.89 bc	78.67 ab
Fermented nettle extract in water (10%)	518.3 bc	11.58 cde	83.11 ab
Fresh nettle extract in cattle urine (20%)	618.7 b	12.20 b	76.00 b
Mancozeb (0.2%)	859.1 a	12.79 a	66.22 c
Control	452.8 c	11.24	87.56 a
CV%	18.97	3.31	11.31

Means within each column followed by same later do not differ significantly by DMRT at $P>0.05$.

Seed infection

Seed infection was also the lowest (66%) in mancozeb sprayed plots, followed by plots sprayed with fresh nettle extract in cattle urine and fresh nettle extract in water at 20% concentration and it was highest in control plots (Table 4). There was, however, no significant difference among nettle extract sprayed plots.

There was a significant interaction between main plot and subplot treatments, with respect to disease index at 117 DAS, 1000 grain weight and seed yield, revealing that increased number of mancozeb sprays reduced disease, which eventually contributed to raise seed yield. The situation was reverse for control (water spray). This may be due to the fact that increased number of water spray increased leaf wetness period (congenial environment for pathogen) that increased disease, which ultimately reduced seed yield.

There was a significant positive correlation between number of alternaria spots on leaves, stem and pod and percent disease index of alternaria blight but a negative correlation between disease and seed yield components. This indicated that the treatments like mancozeb, fresh nettle extract in cattle urine, fresh nettle extract in water and fermented nettle extract in water all at 20% concentration reduced the size and number of spots on leaf, stem and pods (data not shown) resulting in lower disease index on leaves and inflorescence and higher seed yields. This may be due to the fact that blighting of leaves and stems due to *Alternaria* species reduced photosynthetic area resulting in fewer pods/plant and seeds/pod (yield attributing characters). Seed infection by *Alternaria* species caused shriveling of seeds, which reduced 1000 seed weight and seed yield.

CONCLUSIONS

Mancozeb (Dithane® M-45; 75% WP) appeared to be better than nettle extracts for reducing growth of *Alternaria* species under laboratory conditions as well as alternaria blight of radish in field. However, fresh nettle sap extracted in cattle urine at 20% concentration and fresh nettle sap extracted in water at 20% concentration were also effective although fermented extracts were not effective.

In view of certain disadvantages of chemicals, as mentioned above, the nettle extracts have wide scope for commercial exploitation for the management of alternaria blight of radish and other diseases. Detailed studies on formulation of nettle extracts or nettle powder, time and number of applications for management of alternaria blight of vegetable crops are necessary. Further studies on fungitoxic principles and chemical characterization will be helpful in developing commercial botanical fungicides.

ACKNOWLEDGEMENTS

Authors gratefully acknowledge the financial assistance provided by the Seed Sector Support Project (SSSP). We also thank Forum for Rural Welfare and Agricultural Reform for Development (FORWARD) and Institute of Agriculture and Animal Science (IAAS) for necessary management of the study.

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