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Management of Wheat Powdery Mildew using Cow Urine based Plant Extracts

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***Abstract*:** Wheat powdery mildew (PM) caused by *Blumeria graminis* f.sp. *tritici* (*Bgt*) is a disease of global occurrence and importance inflicting huge reduction in yield. Most of the varieties, grown commercially in epidemiologically important areas of India, are susceptible to it and application of fungicides provide a short-term effective means for its management. In present study cow urine-based leaf extracts of eight commonly available plants were evaluated for the management of the disease under *in vitro*, greenhouse and field conditions during 2018-19 at CSK Himachal Pradesh Agricultural University, Palampur, a hot spot for the disease. All the treatments resulted in significantly less spore germination at 5 to 20 per cent concentration as compared with no treatment check. Maximum spore germination inhibition of 78.87, 73.01 and 71.35 per cent was recorded in plant extract of *Azadirachta indica, Calotropis gigantia* and *Justicia adhatoda*, respectively under *in vitro* conditions. The interaction effects of concentration and treatments were significant at 72 hoursof incubation which indicated that treatment effect varied with the concentration. *A. indica* @ 20 per cent was highly effective both as preventive and curative treatment as foliar sprays under greenhouse conditions. The least per cent disease index (PDI) of 19.05 per cent with disease control of 85.28 per cent was recorded in leaf extracts of *C. gigantia* followed by *J. adhatoda* (21.39%) and *A. indica* (25.74%). The highest grain yield of 34.63 q/ha with 39.82 per cent increase over the untreated check was with *C. gigantia* followed by *J. adhatoda* and *A. indica*. The highest net profit of Rs.14649 per ha with cost benefit ratio of 1:5.2.

***Keywords*:** Eco-friendly management, Plant extracts, Wheat, Powdery mildew, *Blumeria graminis tritici*

Powdery mildew (PM), caused by *Blumeria graminis* f.sp. *tritici* (*Bgt*) is a widely prevalent disease of wheat (*Triticum aestivum* L.) causing severe yield losses ranging from 13-34 and 50-100 per cent under low or moderate infection and severe disease pressure, respectively (Basandrai and Basandrai 2017, Vikas et al 2020). The disease has become a potential threat in north western plain zone (NWPZ) and north hill zone (NHZ) with the wide spread cultivation of varieties with powdery mildew resistant gene *Pm8* due to emergence, and fast spread of matching virulences. Most of the present day commercially grown varieties in these areas are susceptible to it (Crop-Protection-Report2022-23_compressed.pdf (aicrpwheatbarleyicar.in)) and there is no breeding program specifically aimed at development of PM resistant varieties. Chemical fungicides have been used successfully for the management of fungal diseases of plants including wheat powdery mildew. However, use of fungicides is being discouraged worldwide due to their hazardous effects on human health, environment, soil, water and development of fungicidal resistant strains of the pathogen (Mehta et al 2022). It is now obligatory and compelling to identify viable alternatives and eco-friendly approaches for the management of various diseases including wheat PM (El-Mougy et al 2004, Naz et al 2018). Extensive research has been undertaken for environmentally safe and easily bio-degradable bio-fungicides and plant extracts. Some of Plant extracts (PEs) have gained popularity and scientific interest for their antibacterial and antifungal activities (Santas et al 2010). Moreover, PEs degrade quickly and have short pre-harvesting intervals and less residual effects (Naz et al 2018). These have been found to be effective in plant disease management and may be safely incorporated as fast-acting and non-pollutive suitable and viable alternative to synthetic fungicides (Joseph et al 2008). The present investigations were undertaken to determine the efficacy of some commonly available plant extracts under north western himalayan areas in controlling wheat PM under *in vitro*, greenhouse and field conditions.

MATERIAL AND METHODS

Leaf extracts of eight commonly available plants i.e., Banna (*Vitex negundo*), Akk (*Calotropis gigantia*), Barhen (*Acorus calamus*), Lantana (*Lantana camara*), Neem (*Azadirachta indica*), Dharek (*Melia azedarach*), Eupatorium (*Eupatorium adenophorum*) and Basooti (*Justicia adhatoda*) were evaluated with recommended fungicide propiconazole and control. The fresh leaves of each plant (4kg each) were crushed properly and mixed in 10 litres each of water and cow urine. The mixture was incubated at ambient temperature (18-20°C) in rust-proof containers and the leaves were sieved out after 15 days of fermentation except in case of neem, where the leaves (10 kg) were put in equal quantity of water (10 litres) and were boiled till the content was reduced to half. Thereafter, 4 litres cow urine was added to the boiled decoction. The decoctions so obtained were was considered as 100 per cent stock solution out of which dilutions of 5, 10, 15, 20 per cent concentrations were used.

***In vitro* evaluation of plant extracts:** Conidiospore germination technique was followed using 5, 10, 15 and 20 per cent concentration of PEs to determine their efficacy to inhibit the germination. PEs were prepared by adding known quantity of stock solution in sterilized distilled water. The fresh and viable conidia were suspended on dry and clean cavity slides having 0.05-0.1ml of each PE at different concentrations. The slides were placed in petri plates lined with filter papers moistened with sterile distilled water and were incubated in BOD incubator at 25±1°C. Observations were recorded on spore germination after 24, 48 and 72 h using research microscope at 40x. The slides with conidia in sterile distilled water served as control. The percent conidial germination and inhibition was calculated (Vincent (1947):

$$\text{Per cent inhibition (PI) of spore germination} = \frac{C-T}{C} \times 100$$

Where, PI - Per cent inhibition; C - Germination of conidia in control; T - Germination of conidia in treatment.

***In vivo* evaluation of Plant Extracts**

Greenhouse: Seedlings of a susceptible wheat var. HPW 155 were raised in 10cm diameter pots filled with garden soil and FYM in the ratio of 10:1. The plant extracts were sprayed @ 15 and 20 per cent conc. as pre (preventive) and post (curative) inoculation applications on 10-day old seedlings (at 1-2 leaf stage). Foliar spray of fungicide propiconazole 25% EC @ 0.1% and water were included as recommended and no spray check, respectively for the comparison. The plants were inoculated by locally available isolate of the *Bgt* by dusting fresh and viable conidia 24h before and after spraying of plant extracts. Each treatment was replicated thrice. The data were recorded on per cent disease severity (Mayee and Datar 1986) 10 days after spraying and per cent disease control was calculated.

Field study: The efficacy of different plant extracts was evaluated under field conditions in the experimental fields of CSK Himachal Pradesh Agricultural University, Palampur during the cropping season 2018-19. The susceptible variety HPW 155 was sown in (5m^2) plots, following recommended agronomical practices (http://www.hillagric.ac.in/extension/dee/pdf_files/Rabi_28-8-09.PDF) under irrigated conditions in randomized block design with three replications. The plant extracts, found effective under *in vitro* and greenhouse conditions, were selected for the evaluation at effective dose (15%) and propiconazole 25% EC @ 0.1% and water sprayed plots were included as recommended checks. The foliar sprays were initiated with the appearance of disease and were repeated fifteen days thereafter. The data were recorded for terminal disease reaction on 50 randomly selected plants per plot using 0-9 scale (Saari and Prescott 1975) and was used to work out per cent disease index given by McKinney (1923):

$$\text{Per cent disease index (PDI)} = \frac{\text{Sum of all disease ratings}}{\text{Total no. of plants observed} \times \text{maximum disease score}} \times 100$$

The per cent disease control (%) was calculated by using following formula:

$$\text{Disease control \%} = \frac{PDI_C - PDI_T}{PDIc} \times 100$$

Where, PDIc = PDI in control plot; PDI_T = PDI in treated plot

The data were also recorded on plot yield after harvesting of the crop and were presented as yield q/ha and per cent increase in yield.

Cost benefit ratio (C:B) was calculated (Reddy et al 2004):

Cost benefit ratio (C:B) = total profit (Rs/ha) / total expenditure (Rs/ha)

Data analysis: The data from *in vitro* and greenhouse experiments were analyzed using a completely randomized design (CRD), while field data followed randomized block design (RBD) and factorial randomized block design (FRBD). After angular transformation, the data were analyzed with CPCS 1 and OP Stat version 4.0.5 software (R Core Team 2021). Duncan's Multiple Range Test in R-Studio version 4.0.5 software (R Core Team 2021) was used to compare means among concentrations, treatments, and their interactions for conidiospore germination and disease severity.

RESULTS AND DISCUSSION

In vitro* evaluation of plant extracts against *Blumeria graminis* f. sp. *tritici: The conidial germination inhibition increased with the enhanced plant extract (PE) concentration. All treatments resulted in significantly less conidial germination ranging between 9.88-44.74 per cent (Table 1) as compare to the control i.e. germination in sterile distilled water (60.20%). However, it was more as compared with the germination in the recommended fungicide check i.e. propiconazole @ 0.1% (9.88%) with corresponding conidial germination inhibition of 96.02 per cent. PEs showed significantly less mean conidiospore germination at 20 percent concentration (30.50%) than at 5, 10, 15 per cent concentration. The minimum mean spore germination of 23.43 per cent was in *A. indica* followed by *C. gigantia* and *J. adhatoda*. The highest conidial germination was in *Eupatorium adenophorum* (44.74%) at all the concentrations followed by *Vitex negundo* (43.93%). Concentration and treatment interaction was non-significant at 24 hours whereas, it was significant at 48 and 72 hours. As has been observed in present studies, Ashlesha and Paul (2017) examined *in vitro* antifungal activity of five plant extracts *viz., Ranunculus muricatus* L., *Vitex negundo* L., *Murraya koenigii* (L.) Sprengel, *Melia azedarach* L. and *Eupatorium* L. against seven fungal phytopathogens namely *Sclerotium rolfsii*, *Fusarium solani* f. sp. *pisi*, *F. oxysporum* f.sp. *pisi*, *Sclerotinia sclerotiorum*, *Rhizoctonia solani*, *Phytophthora nicotianae* var *nicotianae* and *Colletotrichum capsici*. Aqueous extract of *V. negundo* showed significant antifungal activity against *S. rolfsii* (91.3%) and *C. capsici*. which was followed by *M. azedarach* against *P. nicotianae* var *nicotianae* (92.8%) and *Eupatorium* against *P. nicotianae* var *nicotianae* (93.5%) and *C. capsici* (93.2%). Khunt et al (2017) reported that six phyto-extracts showed >50% spore germination inhibition of cumin PM (*Erysiphe polygoni*) at various concentrations after 24, 48 and 72 hours. Significantly, the highest spore germination inhibition (83.05%) was recorded in neem extracts followed by garlic (81.21%) after 72 h at 10 per cent concentration. Meena et al (2019) observed that under *in vitro* conditions neem leaf extract was the most effective against PM of black gram (*Erysiphe polygoni*) with 43.01 to 62.14 per cent spore germination inhibition at 2 and 15 per cent concentration, respectively. Riya (2023) observed that only *Eucalyptus hybrida* was effective in inhibiting the growth of *Xanthomonas campestris* and maximum diametric inhibition zone was 1.85 mm at 20 per cent concentration.

Evaluation of plant extracts in greenhouse: All the treatments resulted in significantly less disease severity as compared with the water spray check (53.74%) (Table 2). Propiconazole 25% EC @ 0.1% resulted in the least disease severity and the highest disease control both in the preventive and curative treatments. Among all plant extracts, *A. indica* and *C. gigantia* were highly effective both as the preventive and curative treatments and as preventive treatments at 15 and 20 per cent concentrations, and resulted in the least disease severity and was followed by *J. adhatoda* and *A. calamus*. In curative treatment, the mean disease severity of 19.49 and 15.92, 20.49 and 16.59 per cent were recorded in *A. indica* and *C. gigantia* with corresponding disease control of 82.05 and 88.21 and 81.03 and 87.18 per cent, respectively at 15 and 20 per concentrations. It was followed by *J. adhatoda* and *A. calamus*. Concentration and treatment interaction was non-significant for both the preventive and curative treatment Rettinassababady et al (2000) also observed that neem seed kernel extracts (5%)

Table 1. Efficacy of urine-basedplant extracts (PEs) on conidiospore germination of *Blumeria graminis* f. sp. *tritici*

Treatment	Conidial germination (%)					Conidial inhibition (%)				
	5%	10%	15%	20%	Mean	5%	10%	15%	20%	Mean
Vitex negundo	51.33 (45.75)*	50.33 (45.17)	47.00 (43.26)	44.00 (41.54)	48.16^{h} (43.93)	31.85	33.18	37.61	41.59	36.06
Calotropis gigantia	23.00 (28.64)	21.66 (27.72)	20.00 (26.54)	16.67 (24.08)	20.33^{c} (26.75)	69.47	71.24	73.45	77.88	73.01
Acorus calamus	33.00 (35.05)	32.66 (34.84)	31.00 (33.82)	28.66 (32.34)	31.33^{e} (34.01)	56.19	56.64	58.84	61.94	58.40
Lantana camara	35.00 (36.26)	34.00 (35.65)	32.66 (34.84)	29.33 (32.78)	32.75^{f} (34.88)	53.54	54.87	56.64	61.06	56.53
Azadirachta indica	18.66 (25.58)	18.00 (25.09)	14.33 (22.24)	12.66 (20.83)	15.91^{b} (23.43)	75.22	76.11	80.97	83.18	78.87
Melia azedarach	38.33 (38.24)	38.00 (38.04)	36.33 (37.05)	33.33 (35.25)	36.50^{g} (37.14)	49.11	49.55	51.77	55.75	51.55
Eupatorium adenophorum	51.33 (45.75)	52.33 (46.32)	50.00 (44.98)	44.67 (41.92)	49.58^{i} (44.74)	31.85	30.53	33.62	40.70	34.18
Justicia adhatoda	26.33 (30.86)	22.00 (27.95)	20.66 (27.02)	17.33 (24.59)	21.58^{d} (27.60)	65.04	70.79	72.57	76.99	71.35
Propiconazole 25% EC (Tilt) @ 0.1%	3.00 (9.88)	3.00 (9.88)	3.00 (9.88)	3.00 (9.88)	3.00^{a} (9.88)	96.02	96.02	96.02	96.02	96.02
Control	75.33 (60.20)	75.33 (60.20)	75.33 (60.20)	75.33 (60.20)	75.33^{j} (60.20)	-	-	-	-	
	35.53^{a}	34.73^{c}	33.03^{b}	30.50^{a}						
LSD (p=0.05)	Concentration=0.73, Treatment=1.15, Concentration X Treatment=2.30									

*Figures in parentheses are arc sine transformed values were highly effective in suppressing *Erysiphe polygoni* in black gram under pot culture experiment. Extracts of ginger, curcuma and giant knot weed (*Reynoutria sachalinensis*) leaves were highly effective for wheat PM management (Vechet and Sera 2015). Dubey (2016) tested aqueous extracts of three plants i.e. *Eucalyptus camaldulensis*, *Allium sativum* and *Azadirachta indica* against *Phytophthora infestans* causing late blight of tomato under net house condition. All the treatments significantly reduced the severity of late blight over untreated control. The most effective treatment was clove extract of *A. sativum* followed by *E. camaldulensis* and *A. indica*. Mishra et al (2017) observed that neem leaf extract resulted in significantly less disease incidence (37.20%) of peas PM (*Erysiphe pisi*) as compared with water sprayed check (51.33). Bankatdas (2019) reported that the foliar spray of azadirachtin (10%) under pot culture conditions resulted in less incidence (34.12%) and intensity (18.29%) of coriander PM followed by turmeric extract (10%) and ginger extract (10%).

Evaluation in field: Field trial was conducted to assess the efficacy of 6 promising PEs @ 15 per cent concentration along with propiconazole 25EC @ 0.1% and water sprayed plots as recommended and no spray checks, respectively. All the test PEs resulted in significantly less disease severity (Table 3) as compared with check plots (58.65%) and increased seed yield compared to untreated check (24.77 q/ha). However, recommended fungicide propiconazole 25% EC @ 0.1% was the most effective with maximum disease control (88.33%) and higher yield (38.50 q/ha). Among the plant extracts, the least percent disease index (PDI) of 19.05 per cent with corresponding disease control of 85.28 per cent, was with PEs of *C. gigantia* and was followed by *J. adhatoda* and *A. indica* with corresponding disease control of 81.73 and 74.11 per cent, respectively. Among the PEs, the highest grain yield of 34.63 q/ha with an increase of 9.86 q/ha i.e. 39.82 per cent increase over check was recorded with *C. gigantia* and was at par with the yield with *J. adhatoda* (32.93 q/ha) and *A. indica* (32.20 q/ha) with 8.16 (32.96%) and 7.43 q/ha (30.00%) increase in yield over the check. The highest 1000 grain weight i.e. 49.33 g was with *J. adhatoda* followed by *C. gigantia* and *A. indica*. The highest net profit of Rs.14649 per ha were recorded in plots sprayed with *C. gigantia* followed by *J. adhatoda* indicating that *C. gigantia* was the most effective in controlling wheat powdery mildew.

Table 2. Efficacy of cow urine based different plant extracts on terminal disease severity of wheat powdery mildew in potted seedlings of var. HPW 155 in greenhouse

Treatment	Disease severity and disease control (%) over check under											
	Preventive treatment at						Curative treatment at					
	15%	20%	Mean	15%	20%	Mean	15%	20%	Mean	15%	20%	Mean
	Disease severity (%)	Disease severity (%)		Disease control (%)	Disease control (%)		Disease severity (%)	Disease severity (%)		Disease control (%)	Disease control (%)	
Vitex negundo	24.33 (29.54)*	21.33 (27.50)	22.83^{d} (28.52)	62.56	67.18	64.87	28.67 (32.35)	25.67 (30.42)	27.16^{f} (31.38)	55.90	60.51	58.21
Calotropis gigantia	10.67 (19.04)	6.67 (14.95)	8.66^{a} (16.99)	83.59	89.74	86.67	12.33 (20.49)	8.33 (16.59)	10.33^{bc} (18.54)	81.03	87.18	84.11
Acorus calamus	16.67 (24.03)	13.33 (21.36)	15.00^{b} (22.70)	74.36	79.49	76.93	15.00 (22.59)	13.33 (21.14)	14.16^{cd} (21.86)	76.92	79.49	78.21
Lantana camara	26.00 (30.63)	23.00 (28.64)	24.50^{d} (29.64)	60.00	64.62	62.31	18.00 (25.07)	16.67 (24.07)	17.33^{d} (24.57)	72.31	74.36	73.34
Azadirachta indica	9.00 (17.43)	7.33 (15.65)	8.16^{a} (16.54)	86.15	88.72	87.44	11.67 (19.49)	7.67 (15.92)	9.66^{b} (17.71)	82.05	88.21	85.13
Melia azedarach	20.67 (27.02)	15.00 (22.77)	17.83^{c} (24.89)	68.21	76.92	72.57	21.67 (27.70)	21.00 (27.21)	21.33^{e} (27.46)	66.67	67.69	67.18
Eupatorium adenophorum	32.67 (34.83)	28.33 (32.14)	30.50^{e} (33.49)	49.74	56.41	53.08	38.33 (38.24)	35.67 (36.65)	37.00^{g} (37.44)	41.03	45.13	43.08
Justicia adhatoda	16.33 (23.81)	14.67 (22.48)	15.50^{bc} (23.15)	74.87	77.44	76.16	12.67 (20.80)	9.00 (17.20)	10.83^{bc} (19.00)	80.51	86.15	83.33
Propiconazole 25% EC (Tilt) @ 0.1%	6.00 (14.14)	6.00 (14.14)	6.00^{a} (14.14)	90.77	90.77	90.77	5.67 (13.75)	5.67 (13.75)	5.66^{a} (13.75)	91.28	91.28	91.28
Control	65.00 (53.74)	65.00 (53.74)	65.00^{f} (53.74)				65.00 (53.74)	65.00 (53.74)	65.00^{h} (53.74)			
LSD (p=0.05)	Concentration=1.10, Treatment=2.47, Concentration X Treatment=NS						Concentration=1.71, Treatment=3.84, Concentration X Treatment=NS					

*Figures in parentheses are arc sine transformed value

Table 3. Efficacy of cow urine based different plant extracts @ 15 per cent as two foliar sprays against wheat powdery mildew var. HPW 155 under field conditions

Treatments	PDI (%)	Disease control (%)	Mean yield (q/ha)	Increase over check (q/ha)	Yield increase (%)	1000 seed wt (g)	Total profit (Rs/ha)	Expenditure (Rs/ha)	Net profit (Rs/ha)	C:B
Acorus calamus	24.44 (29.61)	66.50	30.77	6.00	24.21	47.45	11034	3500	7534	3.15
Lantana camara	32.59 (34.79)	55.33	28.40	3.63	14.65	47.30	6679	3500	3179	1.91
Azadirachta indica	18.89 (25.74)	74.11	32.20	7.43	30.00	48.20	13671	3500	10171	3.91
Melia azedarach	40.37 (39.43)	44.67	28.80	4.03	16.27	45.35	7415	3500	3915	2.12
Calotropis gigantia	10.74 (19.05)	85.28	34.63	9.86	39.82	48.64	18149	3500	14649	5.19
Justicia adhatoda	13.33 (21.39)	81.73	32.93	8.16	32.96	49.33	15021	3500	11521	4.29
Propiconazole 25% EC (Tilt) @ 0.1%	8.52 (16.68)	88.33	38.50	13.73	55.43	49.50	25263	4000	21263	6.32
Control	72.96 (58.65)		24.77			45.61				
CD (p=0.05)	3.22		2.57			1.01				

*Figures in parentheses are arc sine transformed value
Labour cost= Rs 260/man/day (5 man/ha), MSP of wheat= Rs 1840,
Rate of Tilt (Propiconazole 25EC) = Rs 1400, Rate of plant extracts= Rs 30/lt

Dinesh et al (2015) also reported that azadirachtin and NSKE at 5 per cent conc. were the most effective in controlling sunflower PM with the least disease index of 25.78 and 27.56 per cent, respectively in contrast to 83.33 per cent disease index in control. Yadav et al (2017) reported that neem leaf extract @ 10% resulted in the least PM intensity (21.55%) of green gram followed by garlic clove extract @ 10% (22.87%). Similarly, Kubde et al (2020) reported less powdery mildew incidence in chilli sprayed with garlic (37.14%) followed by holy basil, turmeric, NSKE neem, onion and mehndi compared with the no spray check (56.21%).

The ethanolic extract showed the presence of many biologically active molecules such as flavonoids, alkaloids, triterpenoids, steroids, saponins, phenols and glycosides in *Calotropis gigantea*, which had strong antimicrobial activity and serve as plant defense mechanism against fungal plant pathogens (Saratha et al 2010).The phytochemical analysis of leaves extracts of *Justicia adhatoda* revealed the presence of various components such as alkaloids, anthraquinones, flavonoids, saponins, phytosterols, triterpenoids and polyphenols (Jayapriya and Shoba 2015) whereas, *A. indica* has been reported to contain 18 active principles including terpenoids, azadirachtin, azadiradione, epoxy-azadiradione, nimbin, solannin, 6-diacethyl-nimbin amongst others. The inhibitory effect of *A. indica* may be due to the presence of antimicrobial compounds especially azadirachtin (Enyiukwu et al 2014).

CONCLUSION

Among various PEs, *A. indica*, *C. gigantia* and *J. adhatoda* were highly effective under *in vitro*, greenhouse and field conditions in reducing the disease severity of wheat powdery mildew and increase yield. Hence, these may be used as alternative to fungicides for the management of PM. Botanical pesticides integrated with other plant disease management practices i.e. varieties with low level of resistance can reduce human health risks and pollution of environment, water and soil. Hence, these will improve export earnings through reducing chemical residue levels on export commodities. These may be the potential candidates for PM management under organic and natural farming and the weed flora being used to prepare extracts at commercial level may be effectively managed and utilized.

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