



P-ISSN: 2349-8528

E-ISSN: 2321-4902

www.chemijournal.com

IJCS 2020; 8(3): 1497-1501

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Received: 24-03-2020

Accepted: 25-04-2020

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Influence of synthetic and natural fungicides, environmental and physiological factors on the uredospore germination of the pathogen causing rust disease in cowpea

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DOI: <https://doi.org/10.22271/chemi.2020.v8.i3t.9407>

Abstract

Cowpea (*Vigna unguiculata* (L.) Walp.) Is one of the most important pulse crops in Karnataka. Rust disease caused by *Uromyces phaseoli* var. *vignae* (Barclay) Arthur is a major threat for its cultivation causing severe yield loss. Among the six fungicides and ten botanicals, maximum per cent inhibition of germination of uredospores was observed at 50ppm in propiconazole 25%EC (78.87%), followed by hexaconazole 5% EC (77.37%). Whereas, least per cent inhibition of uredospores germination was recorded in treatment with tebuconazole 25% EC at 10ppm (67.17%) followed by difenconazole 25% EC (75.70%). Among different botanicals neem extract at 7.5% showed highest inhibition of 68.06%, however, ginger extract at 2.5% recorded least uredospore germination of 26.10%. In order to know optimum temperature and relative humidity for Urediniospore germination, an experiment was conducted under at temperature between 5-35 °C and relative humidity regimes ranging between 0-100%. The maximum per cent of uredospores germination (80.50%) was recorded at 20°C. however germination did not occur at 0°C and 35°C. When the relative humidity was 100 per cent the per cent germination of uredospores was 83.67 per cent which was maximum. No germination occurred at zero per cent relative humidity. Different nitrogen and Carbon sources were tested at three different concentrations to study the influence of physiological factors on urediniospore germination. Highest germination was recorded in sucrose at 1.5% (36.10%), whereas in case of 0.5% mannitol least germination (15.60%) was obtained. Among the nitrogen sources KNO₃ at 1.5% concentration showed maximum germination of 68.07%, least germination was recorded in NaNO₃ at 0.5% with 18.07% germination.

Keywords: Rust disease, uredospore, germination, fungicides, botanicals

Introduction

Cowpea (*Vigna unguiculata*) is an annual herbaceous legume from the genus *Vigna*. It is adapted to wide ranges of soil and rainfall situations, hence, cultivated in tropical and sub-tropical regions of the world. It is grown throughout India and is well suited to dryland agriculture. It is a popular crop due to its rich sources of high-quality protein of about 24.8 per cent, 1.8 per cent fat and 60.3 per cent carbohydrates. (Ddamulira, G., and Santos, C. 2015) [6]. In addition to protein, the edible leaves and seeds of legumes also contain high levels of dietarily-important mineral nutrients, which are needed for human nutrition and health, especially for overcoming trace element deficiency and promoting brain development. The mineral concentrations are reported to be 142–626 and 60–99 mg.kg⁻¹ for Fe, 49–104 and 44–65 mg.kg⁻¹ Zn, 196–394 and 5–32 mg.kg⁻¹ Mn, 8.6–19.7 and 8.3–14.7 mg.kg⁻¹ Cu and 42–55 and 10–22 mg.kg⁻¹ B in cowpea leaves and seeds, respectively (Belane and Dakora, 2011) [2]. However, cowpea production is suppressed by several pests and diseases. Among them, cowpea rust infections caused by *Uromyces phaseoli* var. *vignae* are the most severe and devastating [M. Chandrashekar, *et al.*, 1989] [4].

Rust can appear on any part of the cowpea plant above ground and spreads rapidly to the middle and upper parts of the host especially during pod formation. Cowpea rust interferes with normal root development and uptake of nutrients by plant roots, resulting in reduced seed size and considerable yield loss (PAN 2014) [13].

Uredospores of most rust fungi (e.g. *Uromyces*, *Puccinia*, *Melampsora*) germinate and grow, directed by the leaf surface toward stomata where they cease growth and develop appressoria directly over the stomatal openings. Subsequently, an infection peg grows from the appressorium on the side adjacent to the leaf surface and penetrates the stomatal aperture (Staples *et al.*, 1985, Wynn 1976)^[16, 18] thus forming a very important infection structure in causing disease.

About 2,000 urediniospores of the pathogen are released per day during the dry season [BSPP, 2017]^[3]. At the present, there is no single cost-efficient control measure to prevent rust infection particularly in different geographical areas [Souza *et al.*, 2013]^[15]. However, Production and germination of propagules and pathogen growth rates are strongly dependent on temperature, relative humidity (RH), and, in the case of foliar pathogens, often leaf wetness (Colhoun 1973; Huber and Gillespie 1992)^[5, 8].

Since spore germination is fundamentally important to the growth and dissemination of most fungi. Fungal spores require nutrients to be present in their environment for germination to occur and for outgrowth into hyphae (Abdel-Rahim AM, Arbab HA. 1985)^[1]. Nitrogen metabolism is required for the synthesis of nucleic acids and proteins, processes that are known to occur early during the course of conidial germination (Oshero N, May GS. 2001 and Taubitz A, Bauer B, Heesemann J, Ebel F. 2007)^[12, 17].

Keeping all these points in view an experiment was designed to know the influence of various external factors on the germination of uredospores.

Materials and Methods

The uredospores were collected from a susceptible variety C-152 by scraping the surface of uredia with a pre sterilized scalpel.

In-vitro evaluation of fungicides and botanicals against the pathogen

Six different fungicides (Table 1), ten plant species (Table 2) were tested for its efficacy on uredospore germination at 10, 25 and 50 ppm and 2.5, 5 and 7.5% concentrations respectively. The botanical extract was prepared by crushing the plant parts with distilled water in 1:1 w/v one drop of fungicide/botanical solution was mixed with a drop of spore suspension in a cavity slide. Each treatment was replicated thrice. The number of spores germinated were observed at 24 hour after incubation under the microscope at 40X magnification. Per cent inhibition of uredospore germination was calculated by using the following formula given by Vincent (1947).

$$PI = \frac{C-T}{C} \times 100$$

Where,

PI = Per cent inhibition of spore germination

C = Germination of spores in control

T = Germination of spores in treatment

Studies on effect of temperature and relative humidity on germination of uredospores

Two drops of uredospore suspension were placed on clean glass cavity slides and then they were incubated at different temperature regimes (Table 3). Similar experiment was conducted at different RH regimes (Table 4). The per cent

germination of uredospores was recorded after 24 hr of incubation.

Studies on effect of different carbon and nitrogen sources on germination of uredospores

The carbon sources viz., sucrose, starch, dextrose, maltose, fructose and mannitol at three different concentrations (0.5%, 0.1% and 1.5%), nitrogen sources viz., potassium nitrate (KNO₃), peptone, sodium nitrate (NaNO₃), ammonium sulphate (NH₄SO₄), ammonium nitrate (NH₄NO₃) and ammonium chloride (NH₄Cl) were evaluated at 3 different concentrations (0.5%, 0.1% and 1.5%) on the germination of uredospores. Then 2 drops of spore suspension were mixed with different concentrations of carbon and nitrogen sources on cavity slides separately. After 24hr. of incubation the per cent germination of uredospore was recorded.

Results and discussion

Effect of fungicides and botanicals on the uredospore germination

Fungicides

The above results revealed that all the fungicides tested exhibited maximum inhibition of uredospores germination at their highest concentration (50ppm) (Table 1). Thus among the six fungicides tested propiconazole 25% EC showed highest inhibition of germination of uredospores at 50ppm (78.87%) followed by hexaconazole 5% EC at 50ppm (77.37%) and mancozeb 75% WP (76.57%) and the highest inhibition of germination of uredospores was recorded in tebuconazole 25% EC (67.17%) at 10ppm followed by difenconazole 25% EC (68.17%) at 10ppm and chlorothalonil 75% WP (68.57%) at 10ppm. The present results are in accordance with Kale (1996)^[9] in the chemical management of cowpea rust where he reported that triazoles like propiconazole, hexaconazole and chlorothalonil were effective in inhibition of uredospores.

Table 1: Effect of fungicides on uredospore germination

Fungicides	Per cent reduction of spore germination		
	Concentration (ppm)		
	10	25	50
Propiconazole (25% EC)	70.85	73.50	78.87
Hexaconazole (5% EC)	69.97	73.30	77.37
Tebuconazole (25% EC)	67.17	71.30	74.50
Chlorothalonil (75% WP)	68.57	72.17	76.37
Mancozeb (75% WP)	69.40	73.23	76.57
Difenconazole (25% EC)	68.17	71.37	75.70
	Fungicides (F)	Concentration (C)	F×C
S. Em±	0.15	0.11	0.26
CD (P=0.01)	0.58	0.41	1.00

Botanicals

Among ten botanicals evaluated the highest per cent inhibition of uredospores (68.06%) was recorded in neem extract at 7.5% followed by tulsi at 7.5% with 64.40% of inhibition. The least per cent inhibition of uredospores was obtained in ginger extract (42.33%) followed by turmeric extract (44.13%). However ginger extract at 2.5% inhibited least uredospore germination of 26.10% followed by turmeric extract at 2.5% with 28.66% inhibition. The neem extract has significant effect in inhibition of uredospores germination compared to other botanicals (Table 2). The results are in accordance with Shabana *et al.* (2017)^[14] who studied the effect of botanicals on germination of uredospores of wheat leaf rust (*Puccinia graminis tritici*) and reported that neem

was effective in inhibiting the spore germination of 68.99 per cent.

Table 2: Effect of different botanicals on uredospore germination

Botanicals	Per cent reduction of spore germination		
	2.5	5.0	7.5
Neem	42.63	55.46	68.06
Garlic	40.76	54.43	61.40
Onion	37.80	53.23	61.33
Parthenium	32.76	50.46	59.40
Marigold	34.50	52.43	59.56
Pongamia	31.80	47.46	57.80
Lantana	35.36	52.96	59.70
Turmeric	28.66	46.00	57.73
Ginger	26.10	43.36	57.53
Tulsi	41.23	54.56	64.40
	Botanicals (B)	Concentration (C)	B×C
S. Em±	0.29	0.16	0.51
CD (P=0.01)	1.10	0.60	1.92

Effect of temperature and relative humidity on the uredospore germination

Temperature

The per cent germination of uredospores at different temperatures varied significantly. The results of the experiment are presented in Table 3. In the present study, it is clear that a good germination of uredospores was observed at temperature between 18-24°C. However, at 20°C temperature maximum per cent of uredospores germination (80.50%) was recorded followed by 22°C (73.66%) and there was also a significant difference between them. At 18°C there was germination of 71.00% and at 24°C it was 66.40 per cent with significant difference between them. However, there was no germination at 0 and 35°C. In other temperatures regimes like 5, 10, 15, 26, 28 and 30°C the uredospores germination of 3.0, 15, 32, 37.23, 30.33 and 8.33 per cent was recorded respectively. The present results are in accordance with Mohamed *et al.*, (1986)^[11] who studied on the germination of uredospores of bean rust caused by *Uromyces viciae-fabae*

and reported that temperature below 10 and above 40 °C did not favour the germination of uredospores. Similarly, Emeran *et al.*, (2005)^[7] also found that temperature of 20°C is more favourable for germination of uredospores on legume (*Uromyces viciae-fabae*). Thus, it can be concluded the temperature range for germination of uredospores was found to be between 18 to 24°C.

Table 3: Effect of temperature levels on germination of uredospores

S. No.	Temperature (°C)	Per cent germination	
1.	0	0.00	(0.34)
2.	5	3.00	(9.98)
3.	10	15.00	(22.79)
4.	15	32.00	(34.45)
5.	18	71.00	(57.42)
6.	20	80.50	(63.80)
7.	22	73.66	(59.13)
8.	24	66.40	(54.58)
9.	26	37.23	(37.61)
10.	28	30.33	(33.42)
11.	30	8.33 (16.78)	
12.	35	0.00	(0.34)
	S. Em±	0.69	
	CD (P=0.01)	2.75	

Note: Figures in parenthesis are arcsine values

Relative humidity

The data (Table 4) revealed that maximum uredospores germination was observed at 100 per cent RH (83.67%) followed by 90 per cent (77.16%), 80 per cent (72.00%), 70 per cent (64.87%) and at 60 per cent (41.00%) and there was a significant difference among them. The least per cent germination was recorded in 0 per cent RH (0.00%) followed by 10 per cent (5.16%) with a significant difference between them. From the above results it can be revealed that, there was increase in per cent germination with the increase in relative humidity. Similar results were obtained by Mohamed *et al.* (1986)^[11] who reported highest per cent germination of uredospores of bean rust caused by *Uromyces appendiculatus* at 95 to 100 per cent relative humidity.

Table 4: Effect of relative humidity levels on germination of uredospores

S. No.	Relative humidity (%)	Per cent germination
1.	0	0.00 (0.34)
2.	10	5.16 (13.13)
3.	20	6.43 (14.69)
4.	30	21.60 (27.70)
5.	40	26.03 (30.68)
6.	50	28.33 (32.16)
7.	60	41.00 (39.82)
8.	70	64.87 (53.66)
9.	80	72.00 (58.06)
10.	90	77.16 (61.46)
11.	100	83.67 (66.17)
	S. Em±	0.681
	CD (P=0.01)	2.71

Note: Figures in parenthesis are arcsine values

Effect of different carbon and nitrogen sources on germination of uredospores

From the study, it was observed that there was a significant difference in the uredospore germination among the different concentrations of carbon sources tested.

Carbon

The results indicate that among the six carbon sources the

highest average germination of uredospores was recorded in sucrose at 1.5% (36.10%) followed by starch at 1.5% (32.87%) and there was a significant difference between them and the least average germination of uredospores was recorded in mannitol at 0.5% (15.60%) followed by fructose 0.5% (16.87%) and maltose 0.5% (18.13%) (Table 5). These findings are in accordance with Khedekar (2012) where he reported that maximum spore germination of 77.30 to 83.03%

in two per cent sucrose solution. This indicates that *U. phaseoli* var. *vignae* is a sugar loving fungus and it requires

sugar for its germination and infection on the host.

Table 5: Effect of different carbon sources on germination of uredospores

S. No.	Carbon sources	Per cent germination of uredospores Concentration (%)		
		0.5	1.0	1.5
1.	Sucrose	20.33	26.50	36.10
2.	Starch	19.77	25.20	32.87
3.	Dextrose	18.30	25.03	30.70
4.	Maltose	18.13	23.40	29.40
5.	Fructose	16.87	21.90	28.30
6.	Mannitol	15.60	20.88	28.10
	Carbon sources	Concentration		Cs×C
	(Cs)	(C)		(Cs)
S. Em±	0.33	0.24	0.58	0.33
CD (P=0.01)	1.28	0.91	2.22	1.28

Nitrogen

Similarly it was observed that, among the six nitrogen sources the highest average germination of uredospores was recorded in potassium nitrate at 1.5% (68.07%) followed by ammonium nitrate at 1.5% (65.77%) and ammonium chloride

at 1.5% (64.17%) and the least average germination of uredospores was recorded in sodium nitrate at 0.5% (18.07%) followed by peptone 0.5% (21.33%) and ammonium sulphate 0.5% (23.17%).

Table 6: Effect of different nitrogen sources on germination of uredospores

S. No.	Nitrogen sources	Per cent germination of uredospores Concentration (%)		
		0.5	1.0	1.5
1.	KNO ₃ (Potassium nitrate)	29.83	51.57	68.07
2.	Peptone	21.33	34.17	57.60
3.	NaNO ₃ (Sodium nitrate)	18.07	33.77	55.90
4.	NH ₄ SO ₄ (Ammonium sulphate)	23.17	37.50	59.57
5.	NH ₄ NO ₃ (Ammonium nitrate)	29.47	48.60	65.77
6.	NH ₄ Cl (Ammonium chloride)	25.90	45.53	64.17
	Nitrogen sources(N)	Concentration(C)		N×C
S. Em±	0.34	0.24	0.60	0.34
CD (P=0.01)	1.33	0.94	2.30	1.33

Conclusion

In the present investigation, effects of different parameters viz, temperature, relative humidity, fungicides, botanicals, carbon and nitrogen source were evaluated *in vitro* for the germination of uredospore of cowpea rust pathogen. The fungicide propiconazole 25% EC was able to inhibit the germination of the spore at 50ppm (78.87%). The next best fungicide observed was hexaconazole 5% EC which recorded 77.3% inhibition at 50 ppm. Whereas, the germination of uredospores was inhibited to a maximum extent by neem extract (68.06%) among the botanicals tested. Temperature and relative humidity has a greater influence on the germination of the uredospores. In this study it was observed at the temperature 20°C and relative humidity of 100% there was a maximum number uredospore germination (80.50% and 83.67%, respectively). Further, The carbon source sucrose at 1.5% and nitrogen source KNO₃ at 1.5% exhibited maximum uredospore germination of 36.10% and 68.07%, respectively.

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