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In Vitro and *in Vivo* Evaluation of Fungal Toxicants for the Control of Cotton Rust Caused by *Phakopsora gossypii* (Arth.) Hirat

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ABSTRACT

The efficacy of six botanicals, seven systemic fungicides and six non-systemic fungicides was determined *in vitro* against *Phakopsora gossypii* (cotton rust) by assessing the percentage inhibition of spore germination. Maximum inhibition (92.61%) of uredospore germination was observed when nimbicidine was used, followed by neem seed kernel extract (NSKE) (91.64%). The least inhibition was noticed in *Tridax procumbence* (80.29%). Among the systemic fungicides evaluated, the maximum percentage inhibition of uredospore germination (93.67%) was observed by hexconazole followed by difenconazole (91.59%) and the least inhibition was noticed with myclobutanil (81.98%). Among the six non-systemic fungicides tested against *Phakopsora gossypii*, the maximum inhibition of uredospore germination (89.17%) was noticed in chlorothalonil followed by SAAF (86.54%), mancozeb (84.20%) and copper oxychloride (80.59%). The least effective fungicide was wettable sulphur, with a mean inhibition of 72.96%. In the integrated management of this cotton disease, the treatments hexaconazole - hexaconazole - hexaconazole followed by hexaconazole - nimbicidine - hexaconazole could effectively manage cotton rust and the highest benefit cost ratio was obtained in hexaconazole - hexaconazole - hexaconazole (2.19) followed by difenconazole – difenconazole - difenconazole (2.07).

Keywords: botanicals, cotton, *Phakopsora gossypii*, fungal toxicants, cotton rust
Abbreviations: BCR, cost benefit ratio; **DAS**, days after seeding; **CICR**, Central Institute for Cotton Research; **ICAR**, Indian Council of Agricultural Research; **MARS**, Main Agricultural Research Station; **NSKE**, neem seed kernel extract; **SAAF**, carbendium + mancozeb

INTRODUCTION

Cotton (*Gossypium* spp.) is the most extensively cultivated fiber crop in the world. Cotton contributes 85% of raw materials to the textile industry. India has a unique distinction of cultivating cotton over 9.37 million ha, accounting for about 33% of the total cotton-growing areas of the world with a 22% share of global production (29.0 million bales). India ranks first in area and second in production after China with an average productivity of 526 kg lint ha^{-1} which is very low when compared to world's average productivity of 767 kg lint ha^{-1}. In India it is grown on 9.37 million ha with a production of 29.0 million bales whereas in Karnataka, cotton occupies an area of 390,000 ha with a production of 900,000 bales and with a productivity of 392 kg lint ha^{-1} (Anonymous 2009; CICR 2009).

The economic importance of cotton is mainly its fiber. Lint is universally used as a textile raw material. Cotton seed is the second most important source of vegetable oil and cotton seed cake is a rich source of high-quality protein for animal feed or, after careful processing, for human food. Cotton seed contains 24% protein while the main component of cotton seed cake forms about 47% of products derived from oil expression and is used for ruminants and as fertilizer. The oil constitutes about 15% of the cotton seed and is used for manufacturing edible oil and other food products. It is also used in manufacturing industrial products such as soap and paints. The hull forms about 40% of the seed and is used mainly as fertilizer or as roughage in stock feed (CICR India 2010).

Cotton plays a significant role in various aspects of the economy of major developing countries. In India, no crop can compete with cotton in terms of value-added processing potential (Hitchings 1984). The quality of Indian cotton is very high, absorbent, durable, extremely fine, lustrous and soft. In India, 70% of the crop is cultivated by small and marginal farmers with almost 60% under rainfed conditions, and since nearly 60 million people are employed in cotton production, processing and supply chain, the Indian government is now looking for ways to improve cotton production in order to boost the economy (James 2004).

Introduction of *Bacillus thuringiensis* (*Bt*) cotton hybrids possessing resistance to American boll worm (*Helicoverpa armigera*) has increased in cotton and, as a result, various diseases have appeared in severe form causing considerable losses in yield. For example, in Madhya Pradesh, the crop was 100% infected by *Leaf curl virus* (LCV). Some of the private hybrids and varieties released earlier were resistant to LCV, but *Bt* cotton was susceptible to LCV. Simultaneously, in the Vidarbha belt of Maharashtra, cotton crops planted over 30,000 ha were widely affected due to the emergence of 'root rot', a disease that is believed to be caused by a mismatch of *Bt* genes relevant in the USA and in India. In another study, a team of Indian government cotton experts noted that *Bt* cotton hybrids were susceptible to diseases like bacterial blight, Alternaria leaf spot and grey mildew, these being the major diseases on cotton identified in central and southern parts of India in 2004 (Ashok 2005).

A study on the incidence of diseases on *Bt* and non-*Bt* cotton carried out by the All-India Coordinated Cotton Improvement Project revealed that both *Bt* and non-*Bt* cotton

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hybrids were equally susceptible to bacterial blight, Alternaria leaf spot and grey mildew. However, the outbreak of Alternaria leaf blight and grey mildew in central and south zones was very significant, especially in hybrids such as 'Bunny' and certain *Bt* hybrids (Anon. 2005). Similarly, an incidence of 18.26% wilt, 16.71% boll rot and 12.32% grey mildew were noticed in 'Naigoan' and 'Nanded' during 2003-04 on MECH-184 *Bt* cotton in addition to the presence of Alternaria leaf spot and bacterial blight (Sharma *et al.* 2005).

Among several diseases, rust has suddenly appeared in an epidemic form and caused up to 30-40% loss in total yield of the majority of *Bt* cotton hybrids cultivated in the region during 2009-2010. Cotton rust, caused by *Phakopsora gossypii* (Arth.) Hirat, occurs in tropical and subtropical cotton-growing areas of the world. The disease is widely distributed and sometimes poses significant yield reduction under favorable weather conditions. The disease was reported to reduce the yield of cotton as much as 24% in Coimbatore (Johnston 1963). Puri *et al.* (1998) reported that the disease appeared in the dry season during December–March and was prevalent in Karnataka, Andhra Pradesh and Gujarat.

The present study aimed to assess the efficacy of botanicals and fungicides under *in vitro* and *in vivo* conditions with the objective of controlling cotton rust.

MATERIALS AND METHODS

A roving survey was carried out in the Northern Karnataka, India to know the incidence and severity of rust during *kharif* 2009-10. Farmers fields in different villages of Devadurga, Sindhnur, Manvi and Raichur taluks of Raichur district; Yelburga taluk of Koppal district; Shahapur, Yadgiri and Jevergi taluks of Gulbarga district; Siruguppa and Bellary taluks of Bellary district were covered under the survey programme. In each village five cotton fields were selected randomly on both sides of the road. In each field, five plants were selected at random and the incidence of the disease was recorded with disease scoring scale of 0-4 (Sheo Raj, 1988), as given here below.

Numerical rating	Leaf area covered (%)
0	Immune, completely free from disease
1	Highly resistant, infection of 0 to 10%
2	Moderately resistant, infection of 11 to 20%
3	Moderately susceptible, infection of 21 to 40%
4	Highly susceptible, infection more than 40%

In total, 10 isolates were collected and their herbaria were prepared. Apart from the herbaria of these isolates, permanent slides of the uredospores of all isolates were prepared. The herbaria and permanent slides were sent for identification to National Centre of Fungal Taxonomy, Inderpuri, New Delhi.

Host range study was made in order to find out the capacity of the pathogen to infect host plants other than cotton. Also, this study helps to assess the survival of the pathogen on the hosts other than cotton. In the present investigation, different host species were tried to study for their reaction to pathogen under glasshouse conditions.

The lower surface of each leaf of different hosts of thirty days old plants was inoculated heavily with rust infected leaves of susceptible varieties by stapler method of incubation. After inoculation, the plants were covered with transparent polythene bag to maintain high humidity for 24 h. The reaction of pathogen on each host species was recorded as 'Infection' (+) or 'No infection' (-) and also average number of rust pustules per leaf and size of pustule were also recorded.

Assessment of fungicidal properties of different plant species on *P. gossypii* uredospore germination

Use of chemical fungicides in the management of diseases has led to new problems in addition to solving the existing problem. Botanicals are an ideal source of low cost, eco-friendly, safe and are indigenously available and hence are suitable in plant protection practices in integrated disease management. Hence, screening plant products for their effective antifungal activity against the pathogen is essential to minimize the use of fungicides and is considered to be one of the best components of integrated disease management practices.

Table 1 List of botanicals used *in vitro* evaluation against *Phakopsora gossypii*.

Botanical name	Local name	Family	Plant part used
Azadirachta indica	Neem tree	Meliaceae	Leaf
Azadirachta indica	NSKE	Meliaceae	Seed
Eucalyptus globulus	Nilgiri	Myrtaceae	Leaf
Tridax procumbens	Vibhuti casa	Asteraceae	Leaf
Prosopis julifora L.	Bellary jali	Mimosaceae	Leaf
Azadirachta indica	Nimbicidine	Meliaceae	Seed

Five botanicals (**Table 1**) were collected and extracted with distilled water using a sterilized pestle and mortar. The extracts were strained through two layers of cheese cloth and finally made up to desired concentrations (5.0, 7.5 and 10.0%; v/v) by adding distilled water. The extraction process was repeated three times for each sample.

Preparation of neem seed kernel extract (NSKE)

The dried neem seed kernels were washed thoroughly and air dried. A known quantity of kernels was weighed and soaked in water for 24 h. Then, the kernels were crushed with a grinder and the extract was filtered serially twice in muslin cloth and made up to 5% (w/v) to which 4 ml of spreader (Teepol Ag) was added. In each replicate one control plot without fungicidal application was maintained.

The fungicides were measured accurately just before spraying and mixed thoroughly with water. Three sprays were given per treatment combination at a 10-day interval. The first spray was given immediately after the appearance of rust pustules on lower leaves of the plant in the experimental field at 90 days after seeding. This was followed by two more sprays. To record the observations, five plants were randomly selected in each treatment and tagged. Disease intensity was recorded at 150, 160 and 170 days after seeding using a 0-4 scale and % disease index was calculated (Wheeler 1969) by using this formula:

% disease index = Sum of numerical ratings/number of plants observed×100/maximum disease rating

The observation on cotton yield was also recorded and expressed as q/ha. The benefit: cost ratio was calculated by taking into account the total cost of cultivation and the yield obtained in each treatment.

Cotton rust uredospores were collected first by scraping the surface of pustules with a clean stainless needle from a susceptible

Table 2 List of fungicides used *in vitro* evaluation against *Phakopsora gossypii*.

Common name	Trade name
Systemic fungicides	
Hexaconazole (TATA RALLIS, MUMBAI)	Contaf 5%EC
Propiconazole (SYNGENTA, PUNE)	Tilt 25%EC
Carbendazim (BAYER, MUMBAI)	Bavistin 50%WP
Difenconazole (SYNGENTA, PUNE)	Score 25%EC
Tridemorph (BASF MUMBAI)	Calixin 80% EC
Myclobutanil (BASF MUMBAI)	Index 10%WP
Tebuconazole (TATA RALLIS, MUMBAI)	Raxil 2%DS
Non-systemic fungicides	
Mancozeb	Dithane M-45 75% WP
Zineb	Indofil Z-78
Carbendium + Mancozeb	SAAF 75% WP
Chlorothalonil	Kavach 75%WP
Copper oxychloride	Blue copper 50%WP
Wettable sulphur	Wettasul 80% WP

Table 3 Treatment combinations.

Treatment	Volume (ml)
H-H-H	0.1-0.1-0.1
D-D-D	0.1-0.1-0.1
N-N-N	3.0-3.0-3.0
NS-NS-NS	5.0-5.0-5.0
H-N-H	0.1-3.0-0.1
H-NS-H	0.1-5.0-0.1
D-N-D	0.1-3.0-0.1
D-NS-D	0.1-5.0-0.1
Control (without fungicide)	-

H, hexaconazole; D, difenconazole; N, nimbicidine; NS, neem seed kernel extract

genotype ('Kanaka') and a uniform suspension of 10 to 20 uredospores/ml of water was prepared. A single drop of uredospore suspension was placed in the wells of a series of cleaned cavity slides (Haiman Changlong Co. Ltd. China) to which a single drop of botanical extract and different fungicides (**Table 2**) were also added to get the required concentrations. A cover slip was placed on the cavity slide and the periphery of the cavity was smeared with Vaseline to prevent contamination and water evaporation. A control was maintained with distilled water. Cavity slides were maintained in Petri dishes provided with moist blotting paper and incubated at 27°C.

After 24 h, observations were made in five microscopic fields for each slide and the total number of spores germinated in each microscopic field was recorded and % germination was calculated. The average of three cavity slides was determined out and % inhibition of uredospore germination was calculated by the following formula (Vincent 1927) for each fungicide:

% inhibition of spore germination = (C - T × 100)/C100

where C = number of uredospores germinated in the control; T = number of uredospores germinated in the treatment.

In vitro evaluation of fungicides against *P. gossypii*

The systemic and non-systemic product formulations were evaluated for their efficacy to inhibit the germination of uredospores using the "slide germination technique" (Gururaj *et al*. 2009). The systemic fungicides were tested at 0.025, 0.05 and 0.075% whereas non-systemic fungicides were tested at 0.05, 0.10 and 0.15% (listed in **Table 2**). Observations were made for five microscopic fields for each cavity and the total number of spores and germinated spores in each microscopic field were recorded and % germination was calculated after 24 h of incubation.

Field evaluation of botanicals and fungicides against *P. gossypii*

A field trial on integrated disease management was carried out at MARS, Raichur during *kharif* (July to August) 2009-2010 under irrigated condition to know the efficacy of two fungicides, namely hexaconazole and difenconazole, botanicals, namely NSKE and nimbicidine which were highly effective *in vitro* when evaluated in combinations against cotton rust. The susceptible variety ('Kanaka') was sown in 5.8 × 6.3 m^2 plots with a 90 × 60 cm spacing in a randomized block design with three replications. In all, there were eight treatments besides a control plot without fungicidal application (**Tables 3, 4**).

Percent disease index (wheeler 1969) (PDI) = sum of numerical ratings/number of plants observed maximum disease rating × 100.

Experimental design and statistical analyses

The experiment was conducted in a randomized block design with six treatments and three replications. The data obtained were statistically analyzed by using ANOVA (Snedecor and Cochran 1967). Significant differences were calculated at $P = 0.01$ using the F-test.

RESULTS AND DISCUSSION

Studies on etiology and spread of disease

1. Symptoms of cotton rust

First rust symptoms appeared on the lower surface with minute spots; 12 DAS they appeared on the upper surface. Sharma and Mehta (1996) observed *P. pachyrhizi* for the first time in six districts of Madhya Pradesh during September 1994 on soybean. Verma *et al.* (2004) observed the symptoms of rust on soybean leaves for the first time in Chhattisgarh. The disease-causing agent was confirmed by cutting the vertical cross-section of infected leaves with the help of camera lucida drawings of uredospores as *P. pachyrhiz.* Schneider *et al*. (2005) reported Asian soybean rust caused by *P. pachyrhizi* Sydow from Hawaii in 1994, Eastern and Southern Africa from 1996-1998 and Nigeria in 2001. Sousa *et al.* (2007) reported that the molecular characterization of the pathogen is possible by polymerase chain reaction (PCR), and specific primers were used for *P. pachyrhizi* and *P. meibomiae*, which are the causal agents of soybean rust. Gollener *et al*. (2009) reported that in the new world, *P. pachyrhizi* was first reported in 1990 and then spread to Hawaii; since 2001, it has been found in South America and in 2004, the pathogen entered continental USA. Disease severity of rust was recorded by using a 0-4 scale and PDI was calculated (**Fig. 1A, 1B, 2, 3**).

The rust pustules appeared as uredia on the leaves as small (1-3 mm), pinkish brown spots with a purple halo. Symptoms also appeared on petioles, stem and boll. The uredia were elongated in shape. There was a decrease in photosynthetic area of leaves due to rust pustules and finally severe defoliation was noticed in the field which resulted in the reduction of cotton yield (**Figs. 4, 5**).

Identification of pathogen

An extensive survey was undertaken during 2008-09 in different districts of northern Karnataka. The affected leaf samples showing typical rust symptoms of cotton were collected during the survey (**Figs. 6, 7**). The identification results indicate that all the isolates showing typical rust symptoms produced uredospores which belong to *P. gossypii* (Arth.) Hirat. Further, the pathogenicity of the causal fungus was proved by employing Koch's postulates. *Puccinia arachidis* in groundnut is known almost exclusively by its uredial stage (Mayee 1987). There are a few records of the occurrence of the telial stage on cultivated groundnut and wild *Arachis* species (Hennen *et al.* 1976). Mayee (1987) made attempts to induce telial formation by modifying environmental factors, but failed. Based on his studies, he reported that uredospores were the main, if not the only, means of rust carry over and dissemination in India. Only the uredial stage of cotton rust occurs in India (Srinivasan 1994) and even though initial stages of the formation of telia have been observed, they have not reached maturity. The matured telial stage occurs in and has been described from Venezuela (Malagutti *et al*. 1972).

Table 4 Details of different fungal toxicants used in the experiment.

Common name	Chemical name	Trade name
Hexaconazole 5% EC	2-(2,4 dichlorophenyl) -1- (1H, 1, 2, 4-triazole-1-yl-hexan-2-01)	Contaf
Difenconazole	*Trans*, *cis*-3-chloro-4-(4-methyl-2-cih-1,2,4-triazole-1 group)	Score
Nimbicidine	A neem kernel-based fungicide	Nimbicidine
Neem seed kernel extract	A neem kernel seed-based fungicide	NSKE

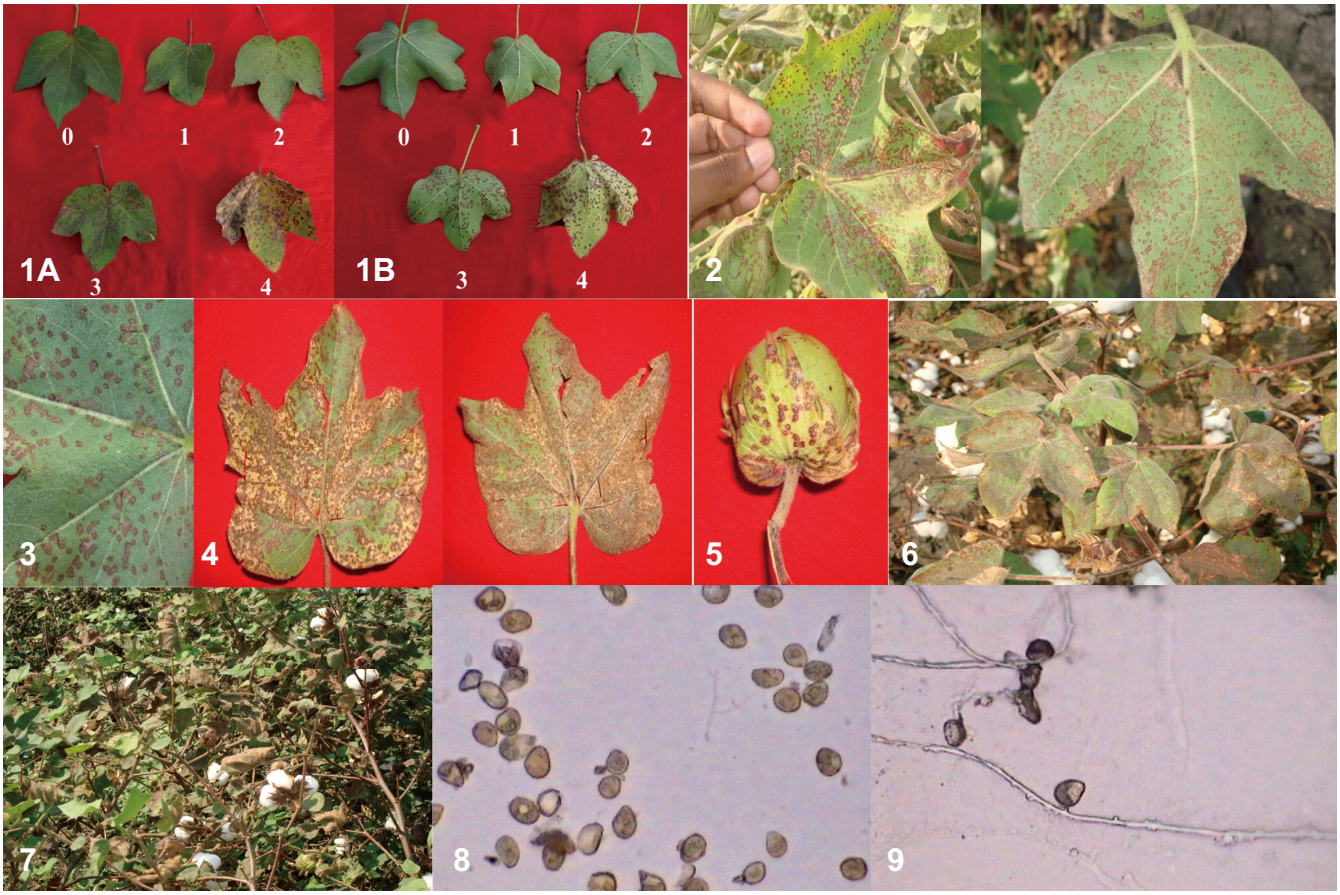

Fig. 1A Scale for scoring cotton rust (adaxial surface). **Fig. 1B** Scale for scoring cotton rust (abaxial surface). **Fig. 2** Typical symptoms of cotton rust on adaxial surface and abaxial surface. **Fig. 3** Closure view of uredial pustules of *Phakopsora gossypii* on cotton leaf. **Fig. 4** Severe symptoms of symptoms of cotton rust on adaxial and abaxial surfaces. **Fig. 5** Rust pustules on cotton boll. **Fig. 6** Severely rust-infected cotton plant. **Fig. 7** Cotton field severely infected with cotton rust. **Fig. 8** Uredeospores of *Phakopsora gossypii.* **Fig. 9** Germination of *Phakopsora gossypii* uredeospores.

Viability and survival of *P. gossypii* uredospores under different storage conditions

Under all storage conditions, there was decrease in % viability of uredospores as time increased. At room conditions, the viability of uredospores was observed up to 40 days of storage which decreased from 83.91 to 4.85%. This was followed by under-tree-shade conditions in which the viability of uredospores remained up to 35 days of storage (81.21 to 6.2%). The viability of uredospores was noticed up to 15 days of storage 5.72, 3.20, 19.22 and 11.32%, respectively). There was no germination of uredospores after 45 days of storage under all storage conditions. Lowest % viability of uredospores (3.2%) was in refrigerator condition stored for 15 days. Patil and Vaishnav (1985) studied the viability of *P. arachidis* uredospores on groundnut plant debris under different storage conditions and noted that the viability of uredospores was lost within a short period (25 days) in open-air conditions and remained viable for a long period (50 days) when stored in a freezer. Patil *et al.* (1997) revealed that *P. pachyrhizi* uredospores, on exposure to natural open conditions (28-30°C), lost their viability within 25 days followed by 28°C under incubator condition for 30 days. The uredospores remained viable for 40 days in a wooden cage outside the laboratory (25-28°C), for 45 days inside laboratory conditions (20-25°C) and for 55 days under tree shade (15-20°C). Hundekar (1999) revealed that uredospores were short lived for 12 to 15 days in the infected host debris under different conditions. Hegde (2001) reported that *P. pachyrhizi* uredospores on exposure to field (28-30°C) and glasshouse (25-28°C) conditions lost their viability within 15 days. Under shade (15-20°C), uredospores remained viable for 35 days and for a longer period (40 days) at room temperature (20-25°C). Gururaj and Srikanth (2007) studied the viability and survival of *P. arachidis* uredospores in groundnut rust and reported that uredospores were viable up to 20 days under field conditions (25-28°C). Park *et al.* (2008) reported the viability of soybean rust uredospores under simulated southern Louisiana winter temperature conditions (12°C, 14-h days and 1°C, 10-h nights with 75% relative humidity) declined rapidly from 72 to 40% after 1 day and then decreased gradually to 17% after 7 days and 11% after 60 days.

Host range studies of *P. gossypii*

A study was carried out to assess the survival of the pathogen through uredospores on different hosts in the absence of the primary host. Rust-infected cotton leaves were stapled to the leaves of various host species raised in a glasshouse and observed for development of symptoms and average number of pustules.

Cotton leaves showed typical pustules of *P. gossypii* indicating that uredospores are viable, infective and that conditions are congenial for infection. Out of 13 host species other than cotton tested, 9 were infected and pustules contained *P. gossypii* uredospores. The hosts (groundnut, okra, pigeon pea, greengram, cowpea, french bean, soybean, sunflower and horse gram) showed rust pustules. However, sesamum, pegionpea, dolichos bean, cluster bean and chickpea did not show any pustules.

do Vale *et al.* (1986) reported that *V. mungo* was the most susceptible host to *P. pachyrhizi* with the highest average number of lesions/cm^2 of leaf area and the highest sporulation intensity. The results are in agreement with Hegde (2001) who reported that pigeon pea, cow pea, French bean, green gram, horse gram, groundnut, cotton, bhendi and sugarcane were found infective to *P. pachyrhizi.* Further, he also reported that French bean and winged bean were found to be more susceptible to the pathogen with

Table 5 *In vitro* evaluation botanicals at different concentrations on % inhibition of uredospore germination of *Phakopsora gossypii*.

Botanicals	% inhibition			Mean
	Concentrations			
	5%	7.5%	10%	
Neem leaf extract	80.20* (63.58)**	85.44 (67.57)	89.20 (70.82)	84.95 (67.32)
NSKE (neem seed kernel extracts)	90.49 (72.05)	91.23 (72.78)	93.19 (74.87)	91.64 (73.23)
Eucalyptus leaf extract	72.23 (58.20)	87.07 (68.93)	89.89 (71.46)	83.06 (66.20)
Tridax procumbens	72.38 (58.30)	82.26 (65.09)	86.23 (68.22)	80.29 (63.87)
Prosopis juliflora	76.15 (60.77)	82.17 (65.03)	89.27 (70.88)	82.53 (65.56)
Nimbicidine	90.50 (72.05)	92.16 (73.74)	95.17 (78.14)	92.61 (74.64)
Mean	80.33 (64.16)	86.72 (68.86)	90.49 (72.40)	85.85 (68.47)

	S.Em±	C.D at 1%
Botanicals (B)	0.14	0.56
Concentration (C)	0.10	0.39
B × C	0.25	0.97

* Figures indicate original values; ** Figures in parenthesis indicate arc-sine transformed values. *F*-test significant at $P < 0.01$.

Table 6 *In vitro* evaluation of systemic fungicides at different concentrations on % inhibition of uredospore germination of *Phakopsora gossypii.*

Botanicals	% inhibition			Mean
	Concentrations			
	0.025%	0.05%	0.075%	
Hexaconazole	90.52* (72.1)**	93.24 (74.93)	97.25 (80.45)	93.67 (75.8)
Propiconazole	75.36 (60.2)	85.24 (67.41)	90.20 (71.75)	83.60 (66.5)
Carbendazim	79.20 (62.9)	83.26 (65.85)	87.25 (69.08)	83.24 (65.9)
Difenconazole	89.24 (70.9)	90.21 (71.76)	95.34 (77.53)	91.59 (73.4)
Tridemorph	86.26 (68.2)	87.25 (69.08)	92.13 (73.70)	88.55 (70.3)
Myclobutanil	75.50 (60.3)	80.22 (63.59)	90.20 (71.76)	81.98 (65.2)
Tebuconazole	74.40 (59.6)	82.48 (65.26)	89.24 (70.85)	82.04 (65.2)

	S.Em±	C.D at 1%
Fungicides (F)	0.05	0.22
Concentration (C)	0.03	0.14
F × C	0.10	0.39

* Figures indicate original values; ** Figures in parenthesis indicate arc-sine transformed values. *F*-test significant at $P < 0.01$.

Table 7 *In vitro* evaluation non- systemic fungicides at different concentrations on % inhibition of uredospore germination of *Phakopsora gossypii.*

Fungicides	% inhibition			Mean
	Concentrations			
	0.025%	0.05%	0.075%	
Mancozeb	80.12* (63.52)**	83.24 (65.83)	89.24 (70.85)	84.20 (66.73)
Zineb	66.27 (54.50)	79.20 (62.86)	85.26 (67.43)	76.91 (61.60)
Carbendazim + Mancozeb (SAAF)	80.18 (63.57)	88.24 (69.95)	91.24 (72.79)	86.54 (68.77)
Chlorothalonil	84.07 (66.48)	90.21 (71.77)	93.25 (74.94)	89.17 (71.06)
Copper oxychloride	76.43 (60.95)	81.08 (64.21)	84.24 (66.61)	80.59 (63.92)
Wettable sulphur	66.39 (54.57)	72.31 (58.25)	80.18 (63.56)	72.96 (58.79)
Mean	75.58 (60.60)	82.37 (65.48)	87.24 (69.36)	81.73 (65.15)

	S.Em±	C.D at 1%
Fungicides (F)	0.05	0.22
Concentration C)	0.03	0.14
F × C	0.10	0.39

* Figures indicate original values; ** Figures in parenthesis indicate arc-sine transformed values. *F*-test significant at $P < 0.01$.

highest average number of pustules/leaf, on the other hand lowest average number of pustules/leaf was recorded in horse gram, sugarcane, cassia, nut grass and stylonthus. However, cotton and soybean were found highly susceptible to the rust pathogen of cotton in the present study. The hosts found to be infective to *P. gossypii* provide scope for the survival of the pathogen in the absence of primary host, hence removal these hosts near to cotton fields would help in avoiding appearance and spread of rust on cotton.

Search for telial stage of *P. gossypii*

An effort was made to search for the telial stage of *P. gossypii* during 2009-2010. Teliospores were not produced or present either on fresh or stored rust samples from infected leaves, stems and petioles collected from different places of northern Karnataka after harvest.

Percentage germination of *P. gossypii* uredospores at different incubation periods

Germination of spores depends on temperature, humidity and incubation period. Hence, uredospore germination at different incubation periods was studied in the laboratory; results are presented in **Table 9**. The germination of uredospores started early (24.21% at 4 h) and it increased over time: 68.20% was recorded after 8 h of incubation. Maximum germination (93.25%) was observed at 72 h of incubation (**Fig. 8**).

Mallaiah and Rao (1979) observed that *P. arachidis* uredospores started to germinate within 2 h and reached a maximum within 6 h. Munde and Mayee (1979) reported

Table 8 Integrated disease management of rust of cotton *kharif* during 2009-2010.

Treatment	% disease index (PDI)				% reduction over control	Yield (q/ha)
	150 DAS	160 DAS	170 DAS	Mean		
H-H-H	12.00 (20.26)*	14.67 (22.50)	19.00 (25.84)	15.22 (22.86)	68.78	18.86
D-D-D	13.33 (21.41)	15.67 (23.31)	20.67 (27.04)	16.67 (23.92)	65.91	18.26
N-N-N	25.00 (30.00)	29.00 (32.58)	35.33 (36.47)	29.89 (33.02)	38.80	13.93
NS-NS-NS	27.00 (31.30)	31.00 (33.83)	37.00 (37.46)	31.67 (34.02)	34.90	13.57
H-N-H	16.00 (23.57)	22.00 (27.97)	24.00 (29.33)	20.67 (26.96)	57.49	16.07
H-NS-H	18.00 (25.10)	23.67 (29.11)	26.33 (30.87)	22.67 (28.36)	53.38	15.88
D-N-D	19.00 (25.84)	23.00 (28.64)	27.00 (31.30)	23.00 (28.60)	52.77	14.91
D-NS-D	21.00 (27.27)	26.00 (30.65)	29.67 (33.00)	25.56 (30.31)	47.43	14.89
Control	34.33 (35.86)	50.67 (45.38)	61.00 (51.36)	48.67 (44.20)	-	10.89
S.Em±	0.48	0.46	0.31	-	-	0.82
CD at 5%	1.45	1.37	0.93	-	-	2.45

*Values in parenthesis are arc sine transformed values
H, hexaconazole; D, difenconazole; N, nimbicidine; NS, neem seed kernel extract

Table 9 Benefit to cost ratio for integrated disease management of cotton rust.

Treatment	Yield (q/ha)	Rs./ha.			Cost of chemical/Kg (Rs.)	Total cost	BC ratio
		Gross returns	Cost of cultivation	Cost of labour			
H-H-H	18.86	56,580	24,594	450	750	25,794	2.19
D-D-D	18.26	54,780	24,474	450	1,500	26,424	2.07
N-N-N	13.93	41,790	23,608	450	900	24,958	1.67
NS-NS-NS	13.57	40,710	23,536	450	1,125	25,111	1.62
H-N-H	16.07	48,210	24,036	450	800	25,286	1.90
H-NS-H	15.88	47,640	23,998	450	875	25,323	1.88
D-N-D	14.91	44,730	23,804	450	1,300	25,554	1.75
D-NS-D	14.89	44,670	23,800	450	1,375	25,625	1.74

that the incubation period increased with an increase in temperature. At 23°C, the incubation period was 6 to 9 days, it was 8 to 10 days at 27°C and 11 to 19 days at 30°C. Further, Benagi (1991) reported that an incubation period of 6 h was required for maximum germination of *P. arachidis* uredospores.

Germination of *P. gossypii* uredospores under different incubation temperatures

Maximum germination (95.62%) of uredospores was recorded after 24 h when stored at 25°C. A higher temperature (30°C) resulted in lower germination (35.10%) at 24 h. Furthermore, % germination of uredospores was most reduced at 35°C (12.89%) and 40°C (1.13%) indicating that temperature above 25°C may not be congenial for uredospore germination (**Fig. 9**).

All the botanicals tested inhibited uredospore germination. Significantly higher inhibition (92.61%) was observed by nimbicidine followed by NSKE (91.64%). The least inhibition was noticed in *Tridax procumbence* (80.29%). Usman *et al*. (1991) found that 2% neem kernel extract was most effective in controlling groundnut rust. Hundekar (1999) reported maximum % inhibition of uredospore germination by tobacco followed by neem and clerodendron. Kadhar (1999) also noticed the maximum inhibition of *P. arachidis* uredospore germination by neem leaf and seed kernel extract at 5% (**Table 5**).

Among the systemic fungicides evaluated, the maximum % inhibition of uredospore germination (93.67%) was observed by hexconazole followed by difenconazole (91.59%); least inhibition was noticed with myclobutanil (81.98%) (**Table 6**). Bengai (1991) evaluated 8 fungicides *in vitro* on *P. arachidis* uredospore germination and reported that propiconazole, with propiconazole at 0.1% effectively inhibited germination.

Among the 6 non-systemic fungicides tested against *P. gossypii*, maximum inhibition of uredospore germination (89.17%) was noticed in chlorothalonil followed by SAAF (86.54%), mancozeb (84.20%) and copper oxychloride (80.59%) (**Table 7**). The least effective fungicide was wettable sulphur, with mean inhibition of 72.96%. Patil (1996) also evaluated 7 systemic and 2 non-systemic fungicides *in vitro* against uredospore germination of *P. helianthi* and found to be more effective even at the lower concentrations tested (0.025 and 0.05%) for the systemic fungicide. The non-systemic fungicides viz., mancozeb and chlorothalonil were found to be effective at higher concentrations (0.1, 0.2 and 0.3%).

All the treatment combinations were significantly superior to the untreated control at each observation Date. Three consecutive applications of hexaconazole and difenconazole resulted in the lowest PDI (15.22 and 16.67, respectively). A combination of hexaconazole alternated with nimbicidine showed a mean PDI of 20.67 followed by combination of difenconazole - nimbicidine - difenconazole (23.00) which did not differ each other but were considerably more effective than the remaining treatments (**Table 8**). Yield differed significantly among the treatments: the untreated control recorded minimum yield (10.89 q/ha) while the plots which received three sprays of hexaconazole and difenconazole produced highest yields (18.86 and 18.26 q/ha, respectively significantly similar). The highest yield was obtained in hexaconazole - hexaconazole - hexaconazole (2.19) followed by difenconazole - difenconazole - difenconazole (2.07) and lowest in a triple application of neem extract (1.62) (**Table 9**). In the present investigation, three consecutive applications of hexaconazole and difenconazole reduced disease severity of rust up to 170 DAS which also reflected on cotton yield. Subrahmanyam *et al*. (1990) also reported that hexaconazole controlled rust and late leaf spot of ground nut best. Sunkad *et al*. (2005) reported that a combination of hexaconazole (triple application) with NSKE controlled rust better (53.93%) than difenconazole (triple application) (53.53%) and another combination (propiconazole - Nimbicidine- propiconazole) (52.37%); these treatments effectively increased pod yield in the same trend that was observed for disease control.

CONCLUSIONS

All the botanicals tested showed the inhibition of uredospore germination of *Phakopsora gossypii.* However, significantly higher inhibition of uredospore was observed in nimbicidine followed by NSKE and least was in *Tridax procumbens*. Among seven systemic and six non-systemic fungicides tested against *Phakopsora gossypii*, hexaconazole and difenconazole among systemic fungicides and chlorothalonil and carbendazim+mancozeb (SAAF) among non-systemic fungicides and were effective in inhibiting uredo-

spore germination of *Phakopsora gossypii.* In the integrated management of the disease, the treatments, hexaconazole - hexaconazole - hexaconazole followed hexaconazole - nimbicide - hexaconazole were found to be effective in managing rust of cotton and increased the yield with maximum BCR. Thus, among the treatment combinations nimbicidine and NSKE intermixed with hexaconazole spray schedule not only reduced the cost of protection but also gave higher benefits.

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