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In vitro efficacy of organics against *A. Candida* causing mustard white rust disease

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Abstract
Phytoextracts and essential oils were evaluated *in vitro* for their efficacy against sporangial germination of *Albugo candida.* Among seven phytoextracts evaluated *in vitro, Azadirachta indica* showed significantly highest sporangial germination inhibition of 53.94% and 57.13%, respectively at 10 and 20 per cent, followed by *Eucalyptus globulus* (50.11% and 56.13%, respectively) and *Ocimum sanctum* (53.23% and 46.26%, respectively). Rest of the phytoextracts tested also showed antifungal activity against the test pathogen. Among the essential oils evaluated *in vitro*, garlic oil resulted with significantly highest sporangial germination inhibition 51.46% and 54.15%, respectively at 500 ppm and 1000 ppm, followed by oils of Neem (51.46% and 54.15%, respectively), Eucalyptus (46.98% and 50.44%, respectively). Rest of the essential oils also significantly inhibited sporangial germination over untreated control.

Keywords: Triclosan, TCS, determination, detection, sensor

Introduction
Rapeseed-mustard is an inevitable component of India's traditional culinary system that can be used as a source of edible oil, raw material for industrial products and as a spice. Major constraints in mustard production are the pests and diseases. White rust is the major and widely prevalent disease of rapeseed and mustard, in India. It is caused by an oomycotic fungi *Albugo candida* (Pers.) Kuntze, which appears in an epiphytotic form, inducing serious damage to the cruciferous crops (Kolte, 1985) [4]. *Albugo* spp. are obligate parasites that reproduce asexually by means of the sporangia/ zoospores and sexually by thick walled oospores. The sporangia are colourless, nearly spherical to rectangular borne on short, 12-18 µm diameter, club-shaped stalks (sporangiophores), each of which produces a chain of spores, in Basipetal succession with distinct thickening between the sporangia.
As sporangia are produced they become tightly packed and eventually rupture the host epidermis. After release, the sporangia are disseminated by air currents, splashing rain, farm implements, workers, and insects. With cool temperatures (below 20 °C) and free water on the host tissue, each sporangium can germinate directly by producing a germ tube or, more commonly, by forming 4 to 18 motile zoospores. The zoospores soon come to rest, become spherical, form a cell wall, lose their flagella, and produce a germ tube. The germ tubes grow and penetrate leaf or other host tissue through stomata (Meena *et al.*, 2014) [7].
With the increased harmful effects of fungicides, the need to utilize organics is gaining importance in recent years. Several organics *viz.*, neem leaf extract, neem oil, garlic extract, eucalyptus leaf extract etc. were earlier reported by several workers as potential alternatives for chemical fungicides against white rust disease (Verma (2005) [9], Kumar (2009) [5], Meena *et al.* (2011) [6], Omranpour *et al.* (2011) [8]. Hence, the present study explores on the *in vitro* efficacy of organics against sporangial germination of *A. candida.*
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Materials and Methods
In vitro efficacy of various fungicides and organics against *A. candida*
Various organics i.e., Phyto extracts and essential oils were evaluated *in vitro* by applying sporangial germination test (glass cavity slides). Sporangial suspension of A. candida was prepared in sterile distilled water and used fresh (within 15 mins. of preparation) for sporangial germination technique. The double strength solutions of each Phyto extracts and essential oils were prepared separately and evaluated their efficacy for inhibition of sporangial germination of the test pathogen. For the purpose, lid and bottom disc of the sterilized glass Petri plates were lined with moistened blotter paper and kept ready to hold the cavity slides.

One drop of each i.e., test Phyto extract / essential oil and sporangial suspension were dispensed in the cavity slides with three cavities in three replications and were placed in bottom disc of glass Petri plates, lined with moistened blotter paper and closed immediately with the lid lined with moistened blotter paper. These Petri plates were then incubated at 25ºC. Simultaneously, alone sporangial suspension in sterile distilled water of test pathogen placed in cavity slide with three cavities and kept in glass Petri plate lined with moistened blotter and incubated at 25ºC were maintained as untreated control.

Observations on spore germination were recorded at 24hrs after incubation, under Research microscope at 40X magnifications, under five different microscopic fields. A sporangium was considered germinated if it lacked cytoplasm and empty (Verma, 1987 and Anonymous, 2012).

The per cent sporangial germination was calculated by following formula (Vincent, 1927) [11]

$$\text{Per cent Germination (PG)} = \frac{A}{B} \times 100$$

PG- Per cent germination

a. Number of sporangia germinated
b. Number of sporangia observed

The per cent inhibition was calculated by the following formula given by Vincent (1927) [11]

$$\text{Per cent Inhibition of sporangial germination (I)} = \frac{C - T}{C} \times 100$$

Germination of sporangia in control
T- Germination of sporangia in treatment.

***In vitro* evaluation of Phyto extracts**

Locally available higher plant species having antimicrobial activities were evaluated *in vitro* (each @ of 10 and 20 %) against *A. candida* by applying sporangial germination test (cavity slide) against the test pathogen.

Experimental details: Phyto extracts (each @ 10 and 20%)
Design: CRD
Replications: Three
Treatments: Eight

Treatment details

Table 1: Phytoextracts

Tr. No	Treatments		Plant parts used
	Scientific Name	Common Name	
T_1	Azadirachta indica	Neem	Leaves
T_2	Lantana camara	Lantana	Leaves
T_3	Ocimum sanctum	Tulsi	Leaves
T_4	Tagetes erecta	Marigold	Leaves
T_5	Chrysanthemum indicum	Chrysanthemum	Leaves
T_6	Curcuma longa	Turmeric	Leaves
T_7	Eucalyptus globulus	Eucalyptus	Leaves
T_8	Control (Untreated)		

Preparation of leaf extract

Leaf extract of test botanicals were prepared by grinding with mixture –cum grinder. The 100 g washed leaves of each botanical were separately macerated in 100 ml distilled water (w/v) and the macerate obtained was filtered through double layered muslin cloth. Each of the filtrate obtained, further filtered through Whitman No.1 filter paper, using funnel and volumetric flask (10 ml). The final clear extracts obtained, were the standard plant extract of 100 per cent concentration, which were evaluated (@ 10 and 20% each) *in vitro* against *A. candida*, by applying sporangial germination test in cavity slide.

***In vitro* evaluation of essential oils**

Locally available essential oils having antimicrobial activities were evaluated *in vitro* (each @ of 500 and 1000 ppm) against *A. candida* by applying sporangial germination test (cavity slide) against the test pathogen.

Experimental details

Essential oils (each @ of 500 and 1000 ppm)

Design: CRD
Replications: Three
Treatments: Eight
Treatment details

Table 2: Essential oils

Tr. No	Treatments	
	Scientific Name	Common Name
T_1	A. indica	Neem oil
T_2	Eucalyptus globulus	Eucalyptus oil
T_3	Syzgium aromaticum	Clove oil
T_4	Cymbopogon citrates	Citronella oil
T_5	Zingiber officinale	Ginger oil
T_6	Allium sativum	Garlic oil
T_7	Cinnamomum verum	Cinnamon oil
T_8	Control (Untreated)	

Results and Discussion

***In vitro* efficacy of Phyto extracts**

All the seven Phyto extracts evaluated *in vitro* (each @ 10% and 20%) were proved antifungal and exhibited significant inhibition of sporangial germination of *A. candida*, over untreated control. The results revealed sporangial inhibition in the range of 28.72% to 53.94% and 35.91% to 57.13%, respectively @ 10% and 20%.

Among Phyto extracts @ 10% concentration, *Azadirachta indica* showed significantly least sporangial germination (28.57%) and highest inhibition over control (53.94%) at 10% concentration, followed by *Eucalyptus globulus* (30.94%, 50.11%, respectively), *Ocimum sanctum* (33.33%, 46.26%,

respectively), *Lantana camara* (35.93%, 42.07%, respectively), *Curcuma longa* (38.97%, 37.17%, respectively), *Tagetes erecta* (40.00%, 35.51%, respectively) and *Chrysanthemum indicum* (44.21%, 28.72%, respectively). Highest sporangial germination observed in untreated control (62.04%). *Curcuma longa* and *Tagetes erecta* were at par with each other with mean sporangial inhibition of 37.17% and 35.51%, respectively.

Table 3: *In vitro* efficacy of Phyto extracts against *A. candida*, causing white rust of mustard

Tr. No	Treatments	10%		20%	
		SG* (%)	Inhibition (%)	SG* (%)	Inhibition (%)
T_1	*Azadirachta indica*	28.57 (32.31)	53.94 (47.26)	27.50 (31.63)	57.13.(49.10)
T_2	*Lantana camara*	35.93 (36.83)	42.07 (40.44)	33.33 (35.26)	48.04 (43.88)
T_3	*Ocimum sanctum*	33.33 (35.26)	46.26 (42.86)	30.00 (33.21)	53.23 (46.85)
T_4	*Tagetes erecta*	40.00 (39.23)	35.51 (36.58)	38.97 (38.63)	39.24 (38.79)
T_5	*Chrysanthemum indicum*	44.21 (41.68)	28.72 (32.41)	41.11 (39.88)	35.91 (36.82)
T_6	*Curcuma longa*	38.97 (38.63)	37.17 (37.57)	35.93 (36.83)	43.99 (41.55)
T_7	*Eucalyptus globulus*	30.94 (33.80)	50.11 (45.06)	28.14 (32.04)	56.13 (48.52)
T_8	Control (untreated)	62.04 (51.97)	0.00 (0.00)	64.15 (53.22)	0.00 (0.00)
S.E. ±		0.32	0.65	0.39	0.68
C.D. (P = 0.01)		1.31	2.69	1.59	2.79

*Mean of three replications, SG: Sporangial Germination
Figures in parenthesis are arc sine transformed values

At 20%, similar trend was observed in respect of sporangial germination and their inhibition. *A. indica* resulted with significantly least sporangial germination (27.50%) and their highest inhibition (57.13%), over untreated control followed by *E. globulus* (28.14%, 56.13%, respectively), *O. sanctum* (30.00%, 53.23%, respectively), *L. camara* (33.33, 48.04%, respectively), *C. longa* (35.93%, 43.99%, respectively), *T. erecta* (38.97%, 39.24%, respectively), and *C. indicum* (41.11%, 35.91%, respectively). The treatments, *Azadirachta indica* (57.13%) and *Eucalyptus globulus* (56.13%) were at par among themselves. Thus, among the Phyto extracts tested, the most effective found were *A. indica, E. globulus* and *O. sanctum.*

The results of present investigation are in concurrence with the earlier findings of several workers. Verma (2005) [9] reported three sprays of *A. indica* as most effective amongst botanicals against mustard white rust, with least disease incidence (27.60%), followed by garlic extract @ 1% (28.20%) and eucalyptus leaf extract @10% (32.00%). Kumar (2009) [5] reported significantly least average mustard white rust disease severity with *E. globulus* (18.3%), followed by neem leaf (19.9%) and garlic clove extract (21.0%). Khodke *et al.* (2016) [3] reported the bio-efficacy of *Eucalyptus globulus,* with significantly higher PDC (38.58%) than neem seed extract (24.28%) against mustard white rust disease.

In vitro efficacy of essential oils

All of the seven essential oils evaluated *in vitro* (each @ 500 and 1000 ppm) exhibited varied antifungal activity against sporangial germination and their inhibition

Table 2: *In vitro* efficacy of essential oils against *A. candida*, causing white rust of mustard

Tr. No	Treatments	500 ppm		1000 ppm	
		SG * (%)	Inhibition (%)	SG* (%)	Inhibition (%)
T_1	Neem oil	30.53 (33.54)	51.46 (45.84)	28.14 (32.04)	54.15 (47.38)
T_2	Eucalyptus oil	33.33 (35.26)	46.98 (43.27)	30.42 (33.47)	50.44 (45.25)
T_3	Clove oil	43.72 (41.39)	30.48 (33.51)	37.82 (37.95)	38.37 (38.27)
T_4	Citronella oil	45.01 (42.14)	28.42 (32.22)	41.11 (39.88)	33.02 (35.07)
T_5	Ginger oil	35.29 (36.45)	43.87 (41.48)	33.33 (35.26)	45.69 (42.53)
T_6	Garlic oil	28.14 (32.04)	55.25 (48.01)	25.00 (30.00)	59.26 (50.34)
T_7	Cinnamon oil	38.14 (38.14)	39.33 (38.84)	35.93 (36.83)	41.45 (40.08)
T_8	Control (untreated)	62.88 (52.46)	0.00 (0.00)	61.38 (51.58)	0.00 (0.00)
S.E.±		0.47	0.59	0.33	0.55
C.D. (P = 0.01)		1.95	2.43	1.38	2.28

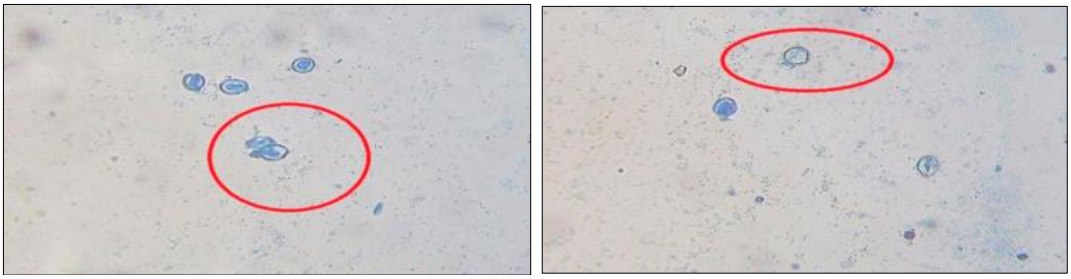
*Mean of three replications, SG: Sporangial Germination
Figures in parenthesis are arc sine transformed values

At 500 ppm Garlic oil resulted with significantly least sporangial germination (28.14%) and highest inhibition of sporangial germination (62.88%), over untreated control followed by the oils of Neem (30.53%, 51.46%, respectively), Eucalyptus (33.33%, 46.98%, respectively), Ginger (35.29%, 43.87%, respectively), Clove (43.72%, 30.48%, respectively) and Citronella (45.01%, 28.42%, respectively). The essential oils of Clove and Citronella were at par among themselves.

Similarly @ 1000 ppm, Garlic oil resulted with significantly least sporangial germination (25.00%) and highest inhibition (61.38%) followed by oils of Neem (28.14%, 54.15% respectively), Eucalyptus (30.42%, 50.44%, respectively), Ginger (33.33%, 45.69%, respectively), Cinnamon (35.93%, 41.45%, respectively), Clove (37.82%, 38.37%, respectively) and Citronella (41.11%, 33.02%, respectively).

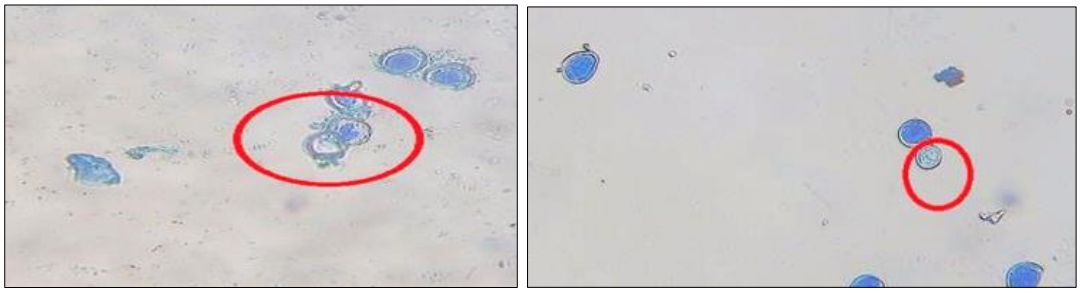
Thus based on antifungal potential, the most effective essential oils in their order of merit were Garlic > Neem > Eucalyptus > Ginger > Cinnamon > Clove > Citronella. Garlic oil, which was found most effective at both 500 and 1000 ppm concentrations, was further used for field evaluation.

These results are in consonance with Kalpana (2017) who reported the efficacy of garlic extract (*Allium sativum*) against *A. candida* @ 100 ppm as effective with highest sporangial germination inhibition (66.79%). Meena *et al.* (2011) [6] reported that under field conditions, seed treatment + foliar sprays of garlic extract @ 1% as most effective with least (17.8%) mustard white rust disease severity in mustard.



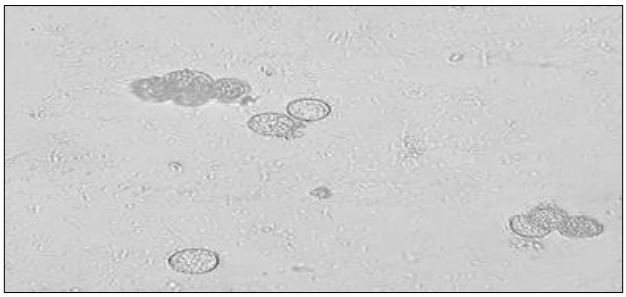
10% **20%**

Neem leaf extract (*Azadirachta indica)*



500 ppm **1000 ppm**

Garlic oil



Control

Fig 1: *In vitro* inhibition of *A. candida* sporangial germination by most effective Phyto extracts and Essential oils

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