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In-vitro assessment of botanicals on the growth and sclerotia production of *Sclerotium rolfsii* Sacc. Causing white rot of onion in Manipur

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Abstract
Five locally available botanicals were evaluated for their effectiveness against growth and sclerotia production of *Sclerotium rolfsii* Sacc. Causing white rot of onion in Manipur. Efficacy of the aqueous plant extracts (1:1 w/v) of these botanicals at four different concentrations were compared with two different concentration of a fungicide Carbendazim by poisoned food technique. Garlic was observed to be most effective and completely inhibited 100% of mycelial growth as well as sclerotia production of the fungus at all the concentrations tested. Sweet flag at 10% inhibited 83.00% and 71.59% of mycelial growth and sclerotia production of the fungus respectively. Wild sage at 10% inhibited 82.33% of mycelial growth of the fungus. Carbendazim at 0.1% concentration inhibited 75.88% and 91.88% of mycelial growth and sclerotia production while Carbendazim at 0.05% inhibited 64.13% and 85.55% of mycelial growth and sclerotia production of the fungus respectively. All the tested botanicals at 10% concentration were effective in inhibiting mycelial growth and sclerotia production of the fungus.

Keywords: Botanicals, *Sclerotium rolfsii* Sacc. White rot, mycelial growth, sclerotia

Introduction
Onion, *Allium cepa* L. is a bulbous crop under the family *Amaryllidaceae.* It was originated in Central Asia (Vavilov, 1951) [28]. Onion is among oldest cultivated plants that are used both as a food and for medicinal applications (Lanzotti, 2006) [15]. The onion crop is attacked by many diseases at different crop stages which not only causes considerable losses in yield but also poses harmful effects during harvesting, post harvesting, processing and marketing stages which reduces the quality and export potential resulting in significant economic losses (Mishra *et al.*, 2014) [18]. White rot of onion caused by *Sclerotium cepivorum* Berk. Was first observed in England (Berkeley, 1841) [2]. The chief mode of dessimination of the disease is through infected onion bulbs and seedlings which are imported for food and propagation purposes (Walker, 1924) [31].

White rot of onion caused by *Sclerotium rolfsii* Sacc. is one of the diseases of economic importance in Manipur. It was found in alarming conditions at various onion growing locations in Manipur. *S. rolfsii* has a broad range of host affecting a large number of both monocotyledonous and dicotyledonous plants (Aycock, 1966, Punja, 1985, El-Nagar *et al.*, 2013) [1, 22, 8]. The fungus produce sclerotia as an overwintering structures that is viable in the soil for more than a year (Punja, 1985, Marcuzzo and Schuller, 2014, Kator *et al.*, 2015) [22, 17, 13]. The fungus also survives as mycelium in infected plants, plant debris and on dead organic materials and sometimes developed hymenial layers for survival (Mullen, 2001) [20]. The extensive use of fungicides has the harmful potential on the environmental and human health and also confers phytotoxic effects on plants (Dias, 2012) [7]. The fungus tends to developed resistance against fungicides. Efficacy of fungicides are variable from year to year, and they are restricted use products (Mullen, 2011) [20]. *S. rolfsii* isolates tolerant to pentachloronitrobenzene (PCNB) were reported from peanut fields in Texas (Shem *et al.*, 1998) [26] and the fungus isolates resistant to tebuconazole, flutolanil and pentachloronitrobenzene (PCNB) had been reported from peanut growing locations in Georgia (Franke *et al.*, 1998) [9].

Hence, in recent years botanicals were extensively tested for their efficacy against plant pathogens as it does not have detrimental effects on environment and human health (Varma and Dubey, 1999) [27]. Extract of botanicals provides an alternative regime for the control of the fungal plant diseases and a promising appreciable choice for a replacement of chemical fungicides (Wongkaew and Sinsiri, 2014) [32]. Therefore, the exploitation and utilization of commonly available local botanicals become essential in management of plant diseases as well as for promotion of organic agriculture. Thus, the present study was undertaken to assess the bio efficacy of aqueous extract of locally available botanicals in the management of white rot of onion caused by *Scerotium rolfsii* Sacc.

Materials and Methods

Isolation and maintenance of the pathogen

The diseased bulbs were collected. The cottony white mycelium from the basal plate of the bulb just adjoining the roots was inoculated on potato dextrose agar and were incubated at 28 ± 1^{0}C for 4 days. The fungus was purified by hyphal tip cut method and re-isolated on PDA. The fungus was examined and identified as *Sclerotium rolfsii* Sacc. On the basis of morphological characteristics and taxonomic keys available in the relevant monographs (Saccardo, 1913; Mordue, 1974) [23, 19]. Culture was maintained on PDA and was sub cultured periodically to freshly prepared PDA throughout the research period.

Collection of locally available botanicals

Five locally available botanicals which are known for their antifungal properties and also easily available in Manipur were chosen for investigating their bio efficacy against *S. rolfsii.* The bio efficacy of these botanicals were compared with two concentrations of a fungicide Carbendazim.

Table 1: List of botanicals screened for their bio efficacy

Serial Number	Botanicals	Scientific Name	Plant parts to be used	Concentrations (%)			
1	Wild sage	*Lantana camara* L.	Leaf	3	5	7	10
2	Garlic	*Allium sativum* L.	Clove	1	1.5	2	3
3	Chaste tree	*Vitex trifolia* L.	Leaf	3	5	7	10
4	Sweet flag	*Acorus calamus* L.	Rhizome	3	5	7	10
5	Neem	*Azadirachta indica* A. Juss	Leaf	3	5	7	10

Preparation of aqueous plant extracts

The collected plant parts were first washed in running tap water and then washed in sterile distilled water. The plant parts were air dried over blotting paper. The air dried plant parts were grinded individually in sterilized mortar and pestle with sterile distilled water at ratio (1:1 (w/v) and were filtered through two fold muslin cloth. The filtrates were centrifuged at 1500 rpm for 15 minutes. The supernatant were collected and were considered as 100% concentration.

Assessment of botanicals against mycelial growth and sclerotia production of the pathogen

Poisoned food technique (Grover and Moore, 1962) [10] was employed for *in-vitro* assessment of the botanicals. Each treatment has three replications. Potato dextrose agar (PDA) was prepared and autoclaved at 121°C at 15lbs for 20 minutes. The first and second concentrations of aqueous plant extract were compared with 0.05% concentration of Carbendazim. The third and fourth concentrations of aqueous plant extract were compared with 0.1% of Carbendazim. Appropriate quantity of individual aqueous plant extracts and a fungicide Carbendazim at the concentrations of 0.05% and 0.1% were added to 50ml molten PDA, shaken thoroughly and were poured into three sterilized petriplates. The molten PDA medium without treatment were poured into three petriplates. This serves as a control. After allowing adequate solidification, each plate was inoculated on the centre with 5mm mycelial disc of a three day old culture of the fungus. The plates were incubated at 28 ± 1^{0}C in inverted position until the control plates were fully grown by the test fungus. Percent inhibition of mycelial growth will be calculated by adopting the method described by Vincent (1927) [29].

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

Where

C = radial growth of the fungus in control

T = radial growth of the growth of the fungus in treatment

Sclerotia produced in each individual aqueous plant extract and in two concentrations of carbendazim will be counted after twenty five days of incubation. Percent inhibition of sclerotia production will also be calculated by adopting the method described by Vincent (1927) [29].

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

Where

C = Number of sclerotia produced by the fungus in control

T = Number of sclerotia produced by the fungus in treatment

Results and Discussion

All the botanicals tested against *Sclerotium rolfsii* inhibited the mycelial growth and sclerotia production of the fungus compared to control. The efficacy of all the botanicals increased with increase in concentrations. Aqueous extract of garlic showed 100% inhibition of mycelial growth and sclerotia production at all the concentrations of 1, 1.5, 2 and 3% respectively. Sweet flag showed 63.35, 71.45, 75.88 and 83.00% mycelial growth inhibition and 66.23, 68.06, 70.29 and 71.59% sclerotia production inhibition at concentrations of 3, 5, 7 and 10% respectively. Wild sage gave 57.77, 69.23, 79.66 and 82.23% mycelial growth inhibition and 43.87, 48.28, 51.57 and 64.04% sclerotia production at 3, 5, 7 and10% concentrations respectively. Carbendazim at 0.1% concentration inhibited 75.88% and 91.88% of mycelial growth and sclerotia production of the fungus. Carbendazim at 0.05% inhibited 64.13% and 85.55% of mycelial growth and sclerotia production of the fungus. No sclerotia were produced by the fungus up to twenty five days of incubation in all concentrations of aqueous garlic extracts. However, the fungus produced sclerotia in case of other plant extracts and carbendazim. The fungus produce lowest number of sclerotia in carbendazim at 0.1% and 0.05% respectively. Chaijuckam and Davis (2010) [5] reported that garlic clove extract and garlic oil completely inhibited mycelial growth and sclerotia

production of *Rhizoctonia oryzae-sativae.* Gupta *et al.* (2012) [11] reported that garlic was most effective and gave 100% inhibition on radial growth of *S. rolfsii* compared to other plant extracts tested against the fungus. Vinod *et al.* (2012) [30] also reported that garlic extract showed maximum inhibitory effects on mycelial growth of *S. rolfsii* and no sclerotia were formed even after 20 days of incubation. Similarly, Darvin (2014) [6] also reported that among several plant extracts tested *in vitro*, clove extract of garlic was most effective against *S. rolfsii* recording lowest mycelial growth (0.0cm) and highest percent inhibition (100%). The effectiveness of garlic against plant pathogens might be attributed due to antifungal activity of allicin, E- and Z-ajoene and iso-E-10-devinylajoene (Prithiviraj *et al.*, 1998) [21]. Sachin *et al.* (2009) [24] reported antifungal potential of dried powder of *Acorus calamus* (sweet flag) against major soil borne pathogens namely, *Rhizoctonia solani, Sclerotium rolfsii, Fusarium solani, F. oxysporum* and *Sclerotinia sclerotiorum.* Jadon and Tiwari (2011) [12] reported that *A. calamus* among all medicinal plant leaf extracts significantly inhibited the growth and sclerotia formation of *S. rolfsii.* Bapat *et al.* (2016) [3] reported the antifungal activity of *A. calamus* against *S. rolfsii* and stated that ethalonic extract of *A. calamus* gave 61% inhibition of mycelial growth and petroleum ether extract of *A. calamus* gave 100% inhibition of mycelial growth of the fungus. He also suggested the use of *A. calamus* as biological fungicide. Saraf *et al.* (2011) [25] reported antifungal activity of *Lanatana camara* (wild sage) against *S. rolfsii* and also reported good percentage growth inhibition of the fungus. Gupta *et al.* (2012) [11] reported that 10% concentration of *L. camara* gave 87.1% inhibition on radial growth of *S. rolfsii.* Kiran *et al.* (2006) [14] reported that 10% concentration of boiled leaf extract of *L. camara* gave 44% inhibition of sclerotia production after 14 days. Bhagat and Chakraborty (2013) [4] reported that higher concentration of Carbendazim was significantly superior over control in checking the mycelial growth of *S. rolfsii* however less inhibitory at 1% and 0.05% concentrations among five systemic fungicides tested against the fungus. Mahato *et al.* (2014) [16] reported that Carbendazim (0.1%) was less effective against *S. rolfsii* compared to other fungicides tested against the fungus and showed 70.44% inhibition on radial growth of the fungus.

Table 2: Effect of aqueous plant extract at first concentration

Treatment	Concentration (%)	Growth (cm)*	Inhibition (%) over control	Sclerotia production*	Per cent inhibition on sclerotia production
Wild sage	3	3.80 (2.07)	57.77	546	43.87
Garlic	1	0.00 (0.71)	100	0	100.00
Chaste tree	3	3.70 (2.05)	58.88	392	59.68
Sweet flag	3	3.30 (1.95)	63.35	329	66.23
Neem	3	4.30 (2.19)	52.23	800	17.86
Carbendazim	0.05	3.23 (1.93)	64.13	140	85.55
Control		9.00 (3.08)	0.00	974	0.00
SE(d) ±		0.05 (0.01)			
$CD_{(0.05)}$		0.12 (0.03)			

*Mean of three replications
Figures in parenthesis are (√x+0.5) transformed values

Table 3: Effect of aqueous plant extract at second concentration

Treatment	Concentration (%)	Growth (cm)*	Inhibition (%) over control	Sclerotia production*	Per cent inhibition on sclerotia production
Wild sage	5	2.77 (1.81)	69.23	503	48.28
Garlic	1.5	0.00 (0.71)	100.00	0.00	100
Chaste tree	5	3.13 (1.91)	65.23	392	59.72
Sweet flag	5	2.57 (1.75)	71.45	311	68.06
Neem	5	4.23 (2.18)	53.00	715	26.55
Carbendazim	0.05	3.23 (1.93)	64.13	140	85.55
Control		9.00 (3.08)	0.00	974	0.00
SE(d) ±		0.06 (0.01)			
$CD_{(0.05)}$		0.15 (0.04)			

*Mean of three replications
Figures in parenthesis are (√x+0.5) transformed values

Table 4: Effect of aqueous plant extract at third concentration

Treatment	Parts used	Concentration (%)	Growth (cm)*	Inhibition (%) over control	Sclerotia production*	Per cent inhibition on sclerotia production
Wild sage	Leaf	7	1.83 (1.53)	79.66	471	51.57
Garlic	Clove	2	0.00 (0.71)	100.00	0	100
Chaste tree	Leaf	7	2.87 (1.83)	68.12	353	63.75
Sweet flag	Rhizome	7	2.17 (1.63)	75.88	289	70.29
Neem	Leaf	7	4.33 (2.20)	51.88	236	75.73
Carbendazim	__	0.1	2.17 (1.63)	75.88	79	91.88
Control			9.00 (3.08)	0.00	974	0.00
SE(d) ±		0.03 (0.01)				
$CD_{(0.05)}$		0.08 (0.02)				

* Mean of three replications
Figures in parenthesis are (√x+0.5) transformed values

Table 5: Effect of aqueous plant extract at fourth concentration

Treatment	Concentration (%)	Growth (cm)*	Inhibition (%) over control	Sclerotia production*	Per cent inhibition on sclerotia formation
Wild sage	10	1.60 (1.45)	82.23	350	64.04
Garlic	3	0.00 (0.71)	100.00	0	100.00
Chaste tree	10	2.50 (1.73)	72.24	321	67.00
Sweet flag	10	1.53 (1.43)	83.00	276	71.59
Neem	10	3.47 (1.99)	61.45	183	81.22
Carbendazim (check)	0.1	2.17 (1.63)	75.88	79	91.88
Control		9.00 (3.08)	0.00	974	0.00
SE(d) ±		0.04 (0.01)			
$CD_{(0.05)}$		0.11 (0.03)			

*Mean of three replications
Figures in parenthesis are (√x+0.5) transformed values

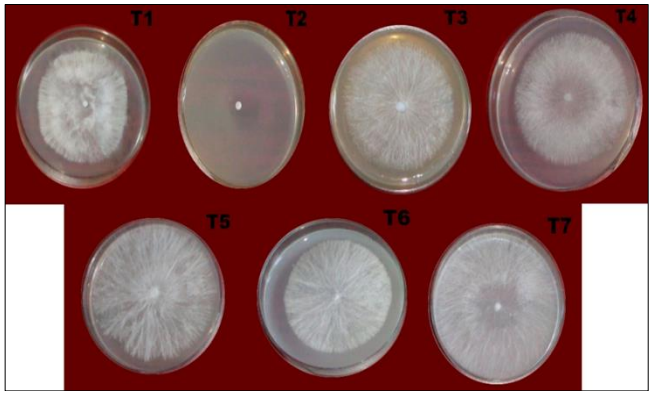

Fig 1: Effect of aqueous plant extracts in comparsion with Carbendazim (0.05%) on the mycelial growth of *S. rolfsii* Sacc. at first concentration. 1. Wild sage 2. Garlic 3. Chaste tree 4. Sweet flag 5. Neem 6. Carbendazim 7. Control

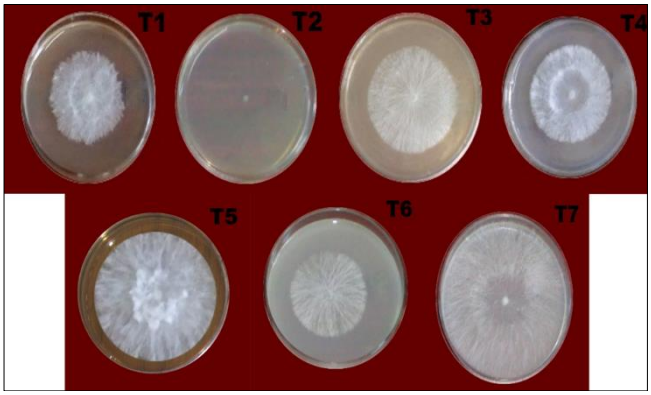

Fig 3: Effect of aqueous plant extracts in comparsion with Carbendazim (0.1%) on the mycelial growth of *S. rolfsii* Sacc. at third concentration. 1. Wild sage 2. Garlic 3. Chaste tree 4. Sweet flag 5. Neem 6. Carbendazim 7. Control

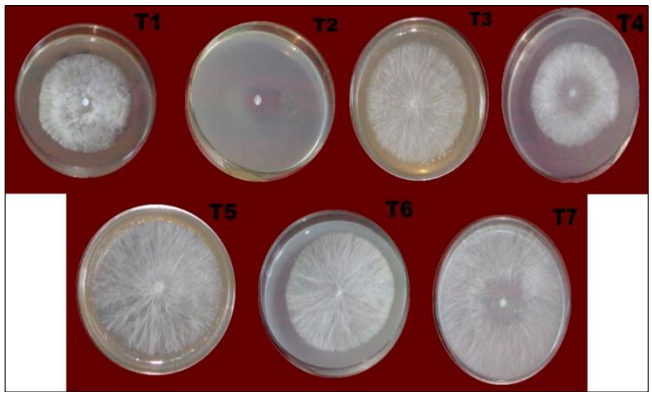

Fig 2: Effect of aqueous plant extracts in comparsion with Carbendazim (0.05%) on the mycelial growth of *S. rolfsii* Sacc. at second concentration. 1. Wild sage 2. Garlic 3. Chaste tree 4. Sweet flag 5. Neem 6. Carbendazim 7. Control

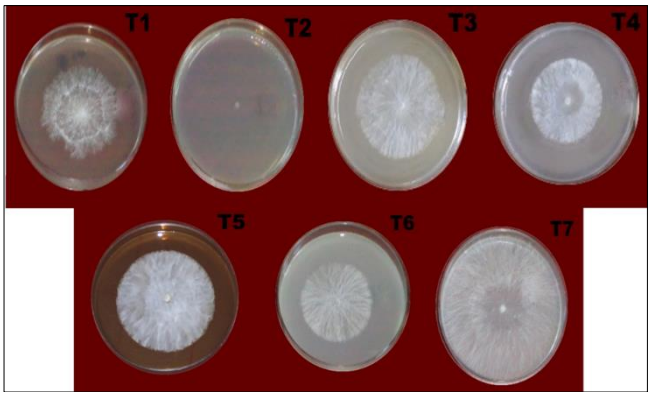

Fig 4: Effect of aqueous plant extracts in comparsion with Carbendazim (0.1%) on the mycelial growth of *S. rolfsii* Sacc. at fourth concentration. 1. Wild sage 2. Garlic 3. Chaste tree 4. Sweet flag 5. Neem 6. Carbendazim 7. Control

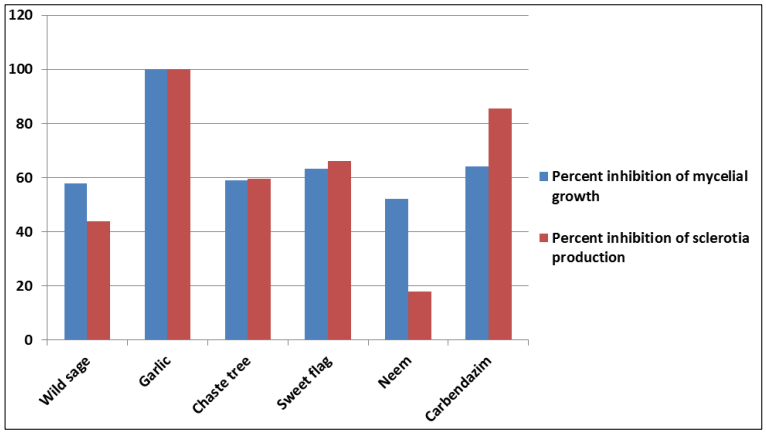

Fig 5: Percent inhibition of mycelial growth and sclerotia production in culture plate at first concentration

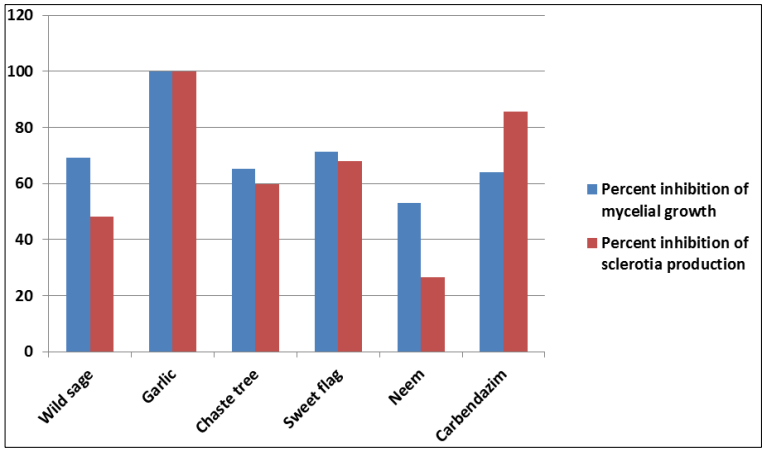

Fig 6: Percent inhibition of mycelial growth and sclerotia production in culture plate at second concentration

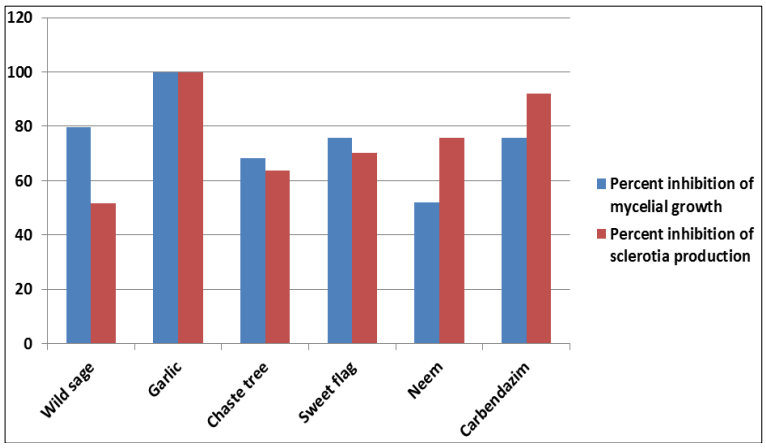

Fig 7: Percent inhibition of mycelial growth and sclerotia production in culture plate at third concentration

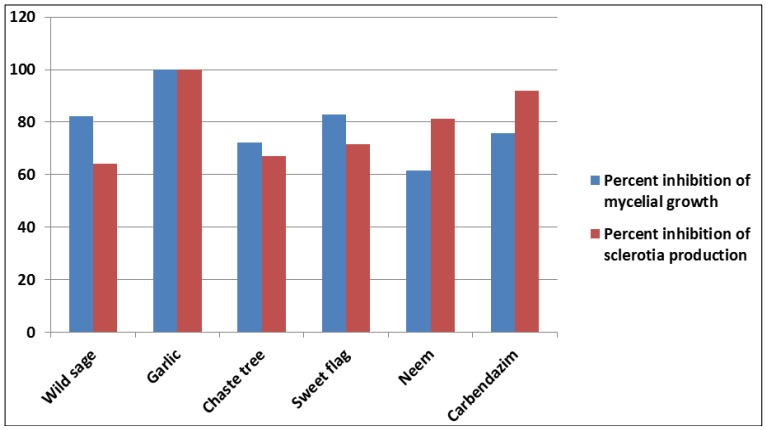

Fig 8: Percent inhibition of mycelial growth and sclerotia production in culture plate at fourth concentration

Conclusion

The present study revealed that locally available botanicals of Manipur tested against *Sclerotium rolfsii* Sacc. Significantly reduced the mycelial growth and sclerotia production of the fungus under laboratory conditions. Since the fungus mostly produced sclerotia as surviving structures, the botanicals that completely inhibits the sclerotia production can be employed as one of a disease management strategies in organic farming or can be incorporated in integrated disease management. Moreover, the effective promising botanicals should also be tested for its bio efficacy under field conditions as the efficacy may vary due to prevailing environmental conditions. Therefore, further research work is considered very essential as there is a rising needs for environmental friendly disease management practices.

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