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Eco-friendly Management of Purple Blotch of Garlic caused by *Alternaria porri* (Ellis) Ciferri

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ABSTRACT: Garlic is second only to onion in popularity among bulb crops and known to suffer from several fungal diseases at various stages of plant growth. Among them, purple blotch caused by *Alternaria porri* is considered to be a devastating disease which causes the yield loss upto the extent of 25-60 per cent under favourable conditions. It is possible to manage purple blotch with the use of fungicides; however, there are a number of problems associated with using them, like environmental pollution, residual effects and killing non-targeted organisms, so its use should be minimized. With a growing demand for good crop health, disease management practices utilizing botanicals, bioagents and indigenous technology knowledge provide a sustainable and safe alternative to chemical pesticides. Hence, the present study is designed to evaluate management practices that are inexpensive and environmentally friendly like use of botanicals, bioagents and indigenous technology knowledge against *Alternaria porri* under laboratory conditions to ensure safe and healthy food. Among seven botanicals tested against *Alternaria porri* by poison food technique, nimbecidine showed maximum mycelial growth inhibition (64.20 %) followed by multineem (59.88 %) at 1 per cent concentration. In case of different bioagents evaluated through dual culture technique, a maximum growth inhibition was observed in *Trichoderma harzianum* (83.89 %) which is followed by *Pseudomonas fluorescens* (63.40 %). In five different indigenous technology knowledge tested by spore germination method, the maximum spore germination inhibition of 81.56 per cent was observed with cow urine followed by jeevamrutha (78.49 %) at 20 per cent concentration. However, the least inhibition of *Alternaria porri* was observed with raw neem oil (20.49 %), *Bacillus subtilis* (38.87%) and vermiwash (29.08 %) among botanicals, bioagents and indigenous technology knowledge, respectively. The eco-friendly treatments, such as bioagents, botanicals and indigenous technology knowledge may help to manage the disease better with minimal use of fungicides.

Keywords: Cow urine, Indigenous technology knowledge, Nimbecidine, *Trichoderma harzianum.*

INTRODUCTION

Garlic (*Allium sativum* L.) is second only to onion in popularity among bulb crops and used as a spice or condiment throughout India. It is an edible and pungent annual bulbous plant in the Amaryllidaceae family. There is constant demand of garlic in the market as it is required daily in small quantity in almost all houses. It has been cultivated in number of countries, due to its vegetative propagation it is susceptible to number of diseases at all stages of plant development. Around the world, downy mildew, rust, purple blotch, stemphylium blight and basal rot have had a significant impact on yields. These diseases also occur during harvesting, post harvesting stages lowering the quality during processing and marketing stages, ultimately reducing the export potential of the crop that significantly causes the qualitative and quantitative economic loss (Prahlad *et al*., 2021). Garlic is most often affected by purple blotch caused by *Alternaria porri* (Ellis) Ciferri (Vijaykumar *et al*., 2021). Purple blotch initially starts as large number of small, whitish circular or irregular spots, smaller than one millimetre diameter. Over time, these spots become larger, increasingly oval-shaped or irregular and turn from white to violet in colour. Afterward, the central part of the spots begins to turn purple accompanied by a pale yellow orange to salmon band extending beyond pale green zone. The dark purple colour is one of the most distinctive characteristics of the disease. The edges of the spots are usually yellow and extend toward the tips and bases of the leaves (Aveling, 1998). The disease is more severe in high humidity levels of 80-90 per cent and moderate

temperature of 25-30 °C. A foliar infection upto 90 per cent has been reported in susceptible cultivars of garlic. Complete damage to the leaf tissues is observed at the time of bulb maturity. Leaves being the only photosynthetic organ directly influence the bulb yield. Significant reduction in bulb yield (25-60 %) due to drying of leaves has been observed in garlic (Bisht and Agarwal 1993). It is possible to manage purple blotch with the use of fungicides; however, there are a number of problems associated with using them like environmental pollution, residual effect and killing non-targeted organisms, its use should be minimized. Eco-friendly management practices such as use of bioagents, botanicals and indigenous technology knowledge (ITK's) proved to be effective in controlling many diseases and also considerably safe to environment and human health. In India, due to purple blotch disease, garlic production has decreased significantly, resulting in an economic loss for the country and ever increasing demand for safe, healthy food in these days, the present investigation has been undertaken.

MATERIAL AND METHODS

The present investigation was carried out during 2019 at the Department of Plant Pathology, College of Agriculture, Dharwad, Karnataka. For analysing the experimental data, arcsine angular transformations were made and analyzed the experimental data in our study for their significance of difference by the usual statistical method adopted for completely randomized designs and we interpreted the results in accordance with Walter (1967). Various botanicals, bioagents and indigenous technology knowledge were evaluated under laboratory condition against *A. porri* through different techniques *viz*., poison food technique, dual culture technique and spore germination method, respectively. For botanicals and bioagents, mycelial growth inhibition was recorded while spore germination was recorded for indigenous technology knowledge. Based on the per cent mycelial growth inhibition and spore germination inhibition, effective organics were identified and those can be used to manage the disease in an eco-friendly way.

Poisoned food technique for the efficacy of botanicals on inhibition of mycelial growth of *Alternaria porri.* Plant based products can be used easily and successfully by farmers against plant pathogenic fungi because of their safety, cheapness and non-hazardous nature. The present study is designed to evaluate the antifungal activity of commercially available botanicals. The following botanicals were evaluated at different concentrations (0.25, 0.5 and 1.0 %) through poison food technique (Nene and Thapliyal, 1982). The required concentrations were prepared by adding known amount of botanical suspension to the melted potato dextrose agar (PDA) media and about 20 ml of poisoned medium was poured in each sterilized Petri plates. No test botanical in the media was served as suitable check. Nine mm mycelial disc of the pathogen was placed in the centre of Petri plate and incubated at 28 ± 1°C. For each treatment, three replications were maintained. Finally the diameter of the colony was measured after reaching maximum growth in control plates. The mycelial growth inhibition per cent was determined through the formula given by Vincent (1947) as follows

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

Where,

I = Mycelial growth inhibition (%).
C = Mycelial growth in control.
T = Mycelial growth in treatment.

Dual culture technique for the efficacy of bioagents on mycelial growth inhibition of *Alternaria porri.* Four bioagents *viz*., *Trichoderma harzianum* Rifai (MH027645.1), *Pseudomonas fluorescens* Migula(NAIMCC-B-01981) and *Bacillus subtilis* (Ehrenberg) Cohn(MT383652.1) were collected from Institute of Organic Farming, UAS, Dharwad and another commercially available *Trichoderma viride* Persoon (Multiplex - market sample) were used to test their bioefficacy against *A. porri* by following dual culture technique (Dennis and Webster 1971).

Twenty ml of sterilized and cooled PDA was poured into sterile Petri plates. Fungal antagonists were evaluated by inoculating the pathogen at one side of the plate and antagonists on the opposite side of the same plate by leaving 3-4 cm gap between them. Bacterial antagonists were streaked in the corner of the plate after which a fungal disk of pathogen was placed. Each treatment was replicated four times with control (without bioagent) maintaining only pathogen. Finally the diameter of the colony was measured after reaching maximum growth in control plates. Per cent mycelial growth inhibition over control was worked out according to the Vincent (1947) formulae as mentioned earlier.

Spore germination method for the efficacy of indigenous technology knowledge on spore germination inhibition of *Alternaria porri.* Five ITK's such as desi cow urine, butter milk (curd diluted in water @ 1:4 and fermented for two days), panchagavya, jeevamrutha and vermiwash were tested against inhibition of spore germination at 5, 10 and 20 per cent concentrations by following spore germination method using cavity slides. Ready-made preparations of panchagavya, jeevamrutha and vermiwash were taken from Institute of Organic Farming (IOF), UAS Dharwad. Required concentrations were prepared by mixing known volume of ITK's in sterile distilled water separately under aseptic conditions. The spore suspension was prepared separately in sterile water to obtain 5×10^6 spores per ml. Then a drop of spore suspension was mixed with one drop of ITK's solution in a cavity slide to achieve the required concentration. A control treatment was maintained with only sterile water. Three replications were maintained for each treatment. Slides were incubated at room temperature (25 ± 1 °C) for 12 hr and observation on spore germination was recorded under compound microscope at 10 X magnification. Spore germination per cent was calculated by following formula

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

Where,

A - Number of germinated conidia

B - Total number of conidia observed

RESULTS AND DISCUSSION

The management of diseases through chemical fungicides leads to various detrimental effects on soil, air and water leading to residual toxicity. Hence, the botanicals, bioagents and indigenous technology knowledge which are easily accessible, non-phytotoxic, renewable, inexhaustible, indigenously available and readily biodegradable, relatively inexpensive and can be utilized in integrated disease management to protect plants. In the present study, different botanicals, bioagents and indigenous technology knowledge were examined under laboratory condition for their antifungal activity against *A. porri*.

Efficacy of botanicals on inhibition of mycelial growth of *Alternaria porri.* Seven test botanicals reduced the mycelial growth of *A. porri* at 0.25, 0.50 and 1.00 per cent concentrations and the data is presented in Table 1 and Plate 1. At 1.00 per cent concentration, nimbecidine inhibited maximum mycelial growth (64.20 %) and found to be the best over other botanicals tested. Next best was multineem which had inhibited mycelial growth upto 42.84, 47.53 and 59.88 per cent at 0.25, 0.50 and 1.00 per cent concentrations, respectively. The least inhibition of mycelial growth was observed in raw neem oil which had recorded 5.43, 18.89 and 20.49 per cent at 0.25, 0.50 and 1.00 per cent concentrations, respectively. Increase in concentration has increased mycelial growth inhibition by all the botanicals tested.

Table 1: Efficacy of commercially available botanicals on mycelial growth inhibition of *Alternaria porri.*

Sr. No.	Commercially available botanicals	Active ingredient	Per cent mycelial growth inhibition			Mean
			Concentrations (%)			
			0.25	0.5	1.0	
1.	Nimbecidine	Azadirachtin 0.03 %	31.48 (34.13)*	43.95 (41.53)*	64.20 (53.25)*	**46.54 (43.02)***
2.	Econeem	Azadirachtin 0.3 %	11.98 (20.25)	36.67 (37.27)	39.75 (39.09)	**29.47 (32.88)**
3.	Neemgold	Azadirachtin 0.15 %	37.16 (37.56)	40.00 (39.23)	54.07 (47.34)	**43.74 (41.41)**
4.	Agroneem	Azadirachtin 1.00 %	28.40 (32.20)	39.88 (39.16)	43.46 (41.24)	**37.24 (37.61)**
5.	Multineem	Azadirachtin 0.03%	42.84 (40.88)	47.53 (43.58)	59.88 (50.70)	**50.08 (45.05)**
6.	Raw neem oil	100 % w/v	5.43 (13.48)	18.89 (25.76)	20.49 (26.92)	**14.94 (22.74)**
7.	Perfekt	Herbal mixture	31.60 (34.21)	38.15 (38.14)	54.57 (47.62)	**41.44 (40.07)**
	Mean		**26.98 (31.30)**	**37.87 (37.98)**	**48.06 (43.89)**	**37.64 (37.84)**
				S.Em. ±		C.D. at 1 %
	Botanicals (B)			0.289		1.128
	Concentrations (C)			0.224		0.874
	B × C			0.501		1.954

* Figures in the parenthesis are arc sine transformed value
Control (only pathogen) recorded 90 mm growth

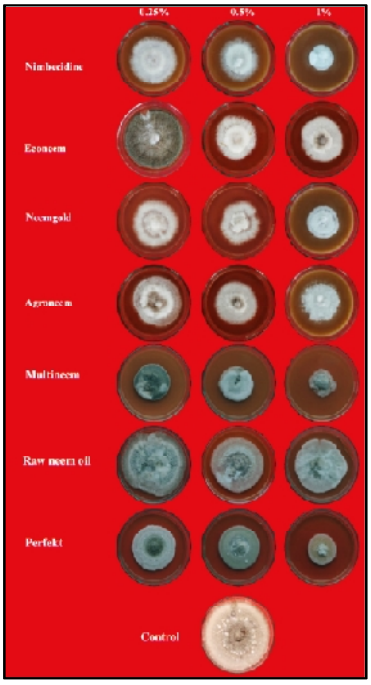


Plate 1: Efficacy of commercially available botanicals on mycelial growth inhibition of *Alternaria porri.*

There was a great deal of variability in the efficacy of botanicals on mycelial growth of *A. porri* under *in vitro*. Significantly higher mycelial growth inhibition was noticed in nimbecidine at 1 per cent concentration (64.20 %). Similar findings were made by Patilkulkarni (2013) while evaluating the bioefficacy of plant extracts against *A. porri* and *Azadirachta indica* found to be the most effective at 10 per cent in inhibiting the mycelial growth of the test pathogen (71.85 %). The most active component of neem is azadirachtin, followed by nimbolinin, nimbin, nimbidin, nimbidol, sodium nimbinate, gedunin, salannin and quercetin. Azadirachtin is a terpene limonoid present in seeds that has properties that are both antifeedant and toxic to pathogens. Through antimicrobial activity, it inhibits microbial growth or potential to break the cell wall of pathogen. Priyanka *et al*. (2017) also studied efficacy of Azadirachtin at 2 and 4 per cent concentrations against *A. porri* and observed 62.09 and 68.61 per cent mycelial growth inhibition, respectively. The results were similar to that of study conducted by Chethana *et al*. (2012); Abdel-Hafez *et al*. (2013) who reported that Azadirachtin compound present in nimbecidine has the capacity to inhibit the mycelial growth of pathogen and it has high potentiality to enter the spore and exhibit the fungi toxicity inturn affecting growth of the pathogen.

Jabeen *et al*. (2013) demonstrated that ethyl acetate fraction of *Azadirachta indica* canretard the mycelial growth of *Alternaria solani*with minimum inhibitory concentration (MIC) of 0.19 mg compared to that of 0.78 g MIC with fungicide (metalaxyl + mancozeb). The results of a study conducted by Shrivastava and Swarnkar (2014) also revealed that methanol and ethanol extracts of neem could inhibitmycelial growth of *Alternaria solani*.

Efficacy of bioagents on inhibition of mycelial growth of *Alternaria porri.* Based on laboratory evaluation of bioagents through dual culture technique, significant differences were observed in per centage of mycelial growth inhibition of *A. porri*. Maximum mycelial growth inhibition of 83.89 per cent was noticed with *Trichoderma harzianum* followed by *Pseudomonas fluorescens* (63.40 %) and *T. viride* (61.20 %). The least mycelial growth inhibition of 38.97 per cent was recorded with *Bacillus subtilis* (Table 2 and Plate 2).

Table 2: Efficacy of bioagents on mycelial growth inhibition of *Alternaria porri.*

Sr. No.	Bioagents	Per cent mycelial growth inhibition
1.	*Trichoderma harzianum*	83.89 **(**66.34**)***
2.	*Trichoderma viride*	61.20 **(**51.47**)**
3.	*Pseudomonas fluorescens*	63.40 **(**52.77**)**
4.	*Bacillus subtilis*	38.87 **(**38.57**)**
	Mean	**61.84 (51.85)**
	S.Em. ±	0.379
	C.D. at 1 %	1.639

* Figures in the parenthesis are arc sine transformed value
Control (only pathogen) recorded 68.30 mm growth

Plate 2: Efficacy of bioagents on mycelial growth inhibition of *Alternaria porri.*

It is possible to reduce inoculum levels of pathogens by using antagonistic microorganisms as a non-chemical means of controlling plant diseases. This would prevent pollution and health hazards associated with fungicides. The competitive ability of antagonists against *Alternaria porri* was studied by dual culture method. Among four bioagents evaluated maximum mycelial inhibition (83.89 %) was noticed in *Trichoderma*

harzianum by over growing on the test fungus. This may simply be a result of the higher competitiveness of *Trichoderma* sp. either through mycoparasitism or antibiosis, and/or because of the possibility of microbial interactions, such as stimulation, inhibition, mutual intermingling of growth of antagonistic isolates over pathogens. It has been reported that *Trichoderma* sp produces secondary metabolites such as 6-phenyl-alpha-pyrone (6pp), derivatives of isocyanide, acids (such as heptelidic acid), peptaibols and enzymes that degrade cell wall proteins (CDWE) which are effective in inhibiting the growth of many phytopathogenic fungi (Vinale *et al*., 2008). Similar findings were observed by Kumari *et al*. (2006); Pramodkumar (2007); Kareem (2008); Rahman *et al*. (2015); Sairam *et al*. (2020) who reported that *T. harzianum* is effective against *A. porri* under *in vitro* condition in inhibiting the maximum mycelial growth of the fungus.

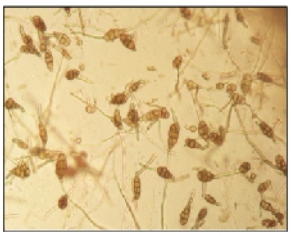
These bioagents likely inhibited pathogen functions by the mechanisms of competition and/or antibiosis. Similar mechanism has been explained by many workers (Mishra and Gupta, 2012; Brahmane *et al*., 2015; Arunakumara *et al*., 2016). Dennis and Webster (1971) made detailed study related to the mechanism of *Trichoderma* sp. and explained that antagonism of this bioagent against many fungi is mainly due to production of acetaldehyde, a carbonyl compound which may be one of the reasons for its antagonistic effect on *A. porri* during the present study.

Efficacy of indigenous technology knowledge on spore germination inhibition of *Alternaria porri.* Five test ITK's reduced the spore germination of *A. porri* at 5, 10 and 20 per cent concentrations and obtained results are presented in the Table 3 and Plate 3. Cow urine, at 5 per cent concentration inhibited the maximum spore germination (40.66 %) and found to be superior among all other ITK's tested. Next best was panchagavya with 37.59 per cent spore germination inhibition and found on par with jeevamrutha (36.17 %). The least spore germination inhibition was noticed in vermiwash (14.89 %) followed butter milk (16.55 %) and found on par with each other. At 10 per cent concentration, maximum spore germination inhibition was noticed in cow urine (72.34 %) which was significantly superior over other ITK's tested. Next best was jeevamrutha (69.27 %) followed by panchagavya (65.48 %) whereas, least spore germination inhibition was noticed in vermiwash (20.33 %). Significant inhibition of spore germination was observed with cow urine at 20 per cent concentration (81.56 %) compared to other ITK's tested. Next best was jeevamrutha (78.49 %) followed by panchagavya (74.94 %) whereas, least spore germination inhibition was noticed in vermiwash with 29.08 per cent.

Irrespective of ITK's concentrations tested, cow urine (64.85 %) found to be the best in inhibiting the spore germination of *A. porri* and found be the best ITK's among all others tested. However, the mean least spore germination inhibition was noticed in butter milk (28.68 %).

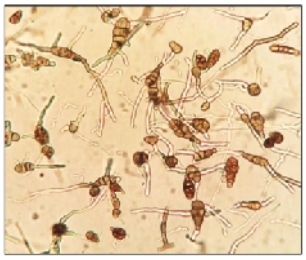
People have developed a number of farming methods during the long journey from primitive agriculture to modern farming through trial and error in their attempts to overcome numerous difficulties they faced during the farming process. Based on generations of experience, this knowledge is derived from close interaction with natural and physical micro-environments. This form of knowledge in today's parlance is popularly known as Indigenous Technology knowledge (ITK) or Indigenous Knowledge System (IKS) and play major role in plant disease management strategy of sustainable agriculture.

In present investigation five different ITK's (cow urine, butter milk, panchagavya, vermiwash and jeevamrutha) are evaluated against spore germination of *A. porri* at different concentrations (5, 10 and 20 %) and their efficacy varied from 29.08 (vermiwash) to 81.56 per cent (cow urine) at 20 per cent concentration. However, the highestspore germination inhibition was noticed in cow urine (81.56 %) followed by jeevamrutha (79.94 %). Cow urine is a product of a cow that has many benefits and is nontoxic. Cow urine (gomutra) is a substance/secretion of animal origin that is claimed to have uncountable therapeutic benefits in Ayurvedic texts.In cow urine, 95 per cent of it is water, 2.5 per cent urea, minerals, 24 salts, hormones, and 2.5 per cent enzymes. Additionally it contains calcium, iron, phosphorus, nitrogen, ammonia, carbonic acid, manganese, sulphur, phosphates, potassium, amino acids, enzymes, cytokine and lactose. Bhadauria (2002) found that cow's urine can be as effective as standard drugs against a wide range of diseases caused by plant pathogens, including ofloxacin, cefpodoxime, gentamycin, and amphotericin B. Several phenolic acids (gallic acid, caffeic acid, ferulic acid, o-coumaric acid, cinnamic acid and salicylic acid) found in cow urine may have inhibited the pathogen's spore germination. The obtained results are in line with the study conducted by Deshmukh *et al*. (2012) who evaluated the antifungal activity of cow urine at different concentrations against wide range of plant pathogens and found that cow urine at 10 and 20 per cent concentrations were more effective in inhibiting the spore germination of *Alternaria solani*. Garg and Kumhar (2020) evaluated cow urine against *A. solani* at four different concentrations (5, 10, 15 and 20 %) under *in vitro* condition. Their results revealed that cow urine (20 %) was the most effective in inhibiting the mycelial growth (78.49 %) than other concentrations tested. The results are in confirmation with the works of Sumangala and Patil (2009); Sharma *et al*. (2010); Pandia *et al*. (2019) who worked on antifungal activity of cow urine against *Alternaria* sp. on different crops and recorded spore germination inhibition at different concentrations.

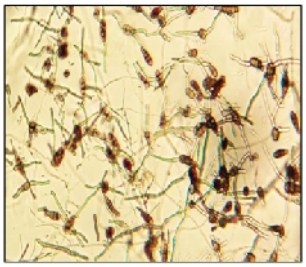


(a) Cow urine @ 20 per cent

(b) Panchagavya @ 20 per cent



(c) Vermiwash @ 20 per cent



(d) Control (sterile distilled water)

Plate 3: Inhibition of spore germination of *Alternaria porri in* different ITK's

Table 3: Efficacy of indigenous technology knowledge on inhibition of conidial germination of *Alternaria porri.*

Sr. No.	Indigenous technology knowledge (ITK's)	Per cent conidial germination inhibition			Mean
		Concentrations (%)			
		5	10	20	
1.	Butter milk	16.55 (24.00)*	27.42 (31.58)*	42.08 (40.44)*	**28.68 (32.38)***
2.	Cow urine	40.66 (39.62)	72.34 (58.27)	81.56 (64.57)	**64.85 (53.64)**
3.	Panchagavya	37.59 (37.81)	65.48 (54.02)	74.94 (59.96)	**59.34 (50.38)**
4.	Vermiwash	14.89 (22.70)	20.33 (26.80)	29.08 (32.63)	**21.43 (27.58)**
5.	Jeevamrutha	36.17 (36.97)	69.27 (56.33)	78.49 (62.37)	**61.31 (51.54)**
	Mean	**29.17 (32.69)**	**50.97 (45.56)**	**61.23 (51.49)**	**47.12 (43.35)**
			S.Em. ±		C.D. at 1 %
ITKs (I)			0.691		2.695
Concentrations (C)			0.536		2.087
I × C			1.197		4.668

*Figures in the parenthesis are arc sine transformed value; Control (sterile distilled water) recorded 94 per cent spore germination

CONCLUSION

Use of botanicals, bioagents and indigenous technology knowledge proved to be effective in inhibiting the pathogen's growth and their application considerably safe to environment and human health. Nimbecidine has shown superiority over other six botanicals in arresting the mycelial growth of *A. porri* (64.20 %) followed by multineem (59.88 %) whereas; least inhibition was observed with raw neem oil (20.49 %) at 1.00 per cent concentration. Among the four bioagents tested, maximum inhibition was noticed with *Trichoderma harzianum* (83.89 %) followed by followed by *Pseudomonas fluorescens* (63.40 %). Irrespective of their concentrations (5, 10 and 20 %) cow urine was significantly superior in inhibiting the spore germination (64.85 %) of *A. porri* over other four ITK's tested. The least spore germination inhibition was noticed in vermiwash (21.43 %).

FUTURE SCOPE

A variety of bioagents, botanicals and indigenous technologies are now being developed and are available worldwide for the use as alternatives to more hazardous and environmentally unacceptable chemical fungicides. Using bioagents, botanicals, and indigenous technologies in conjunction with plant disease management practices can reduce health risks and reduce costs from chemical poisoning and improve export earnings from the reduction of chemical residue levels on export commodities. There are also benefits garnered from preserving natural enemies in crop systems as well as from maintaining indigenous biodiversity. The practical applicability of the bioagents, botanicals and indigenous technologies must be assessed in the field and should be evaluated in conjunction with other management strategies used for the plant disease management.

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Conflict of Interest. None.

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