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Research Article

Management strategy for leaf blight of cabbage (*Brassica oleracea* var. *capitata* L.) in foothills of Nagaland

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Abstract
Cabbage (*Brassica oleracea* var. *capitata* L.) is a major cole crop in India. Cabbage leaf blight caused by *Alternaria* sp. is one of the serious foliar diseases responsible for low production and productivity. The disease is challenging to manage alone with fungicides or bioagents. Hence, the present study was undertaken to manage the disease effectively by utilizing bioagents, fungicides, and their combinations. The field trial was carried out in the Horticultural Research Farm, School of Agricultural Sciences, Nagaland University, Medziphema, Nagaland, during *Rabi*, 2022-2023 and *in vitro* evaluation of fungicides and bioagents conducted in the Department of Plant Pathology, School of Agricultural Sciences, Nagaland University, Medziphema, Nagaland. Management of leaf blight of cabbage was conducted with seven treatments (T_1-T_7). Among the different treatments tested in field condition, the minimum severity of *Alternaria* leaf blight (37.69%) coupled with maximum yield (23.39 t/ha) with a higher-cost benefit ratio of 2.72 were recorded in T_6 (seed treatment with captan @ 3 g/kg + four foliar sprays of azoxystrobin 4.8% + chlorothalonil @ 0.25%). Among the six treatments, *In vitro* evaluation of bioagents *Trichoderma* sp. (TTV-2; T_5) showed maximum inhibition with 69.44%, followed by *Bacillus subtilis* (CRB-7; T_6) with 31.11% inhibition and among the chemical fungicides, azoxystrobin (amistar) showed the highest inhibition of 76.67% as compared to control. These treatments can effectively manage cabbage leaf blight disease and ensure sustained yields, particularly in the foothills of Nagaland.

Keywords: *Alternaria* leaf blight, Cabbage, Disease management, Percent disease index

INTRODUCTION

Cabbage is the world's most popular cole crop because of its high-quality taste, flavour and nutritional content. It has grown widely throughout the year. India's share is 11.95% of vegetables in total global production (FAO, 2023). The production of vegetables in India is 204.84 million tonnes on 11.35 million hectares (DoAFW, 2023). Fungal and insect pests pose a significant threat to *Brassica* crops, while bacterial and viral pathogens have a negligible impact on productivity (Abdel-Farida *et al*., 2009). The most common and destructive disease of cabbage is leaf blight caused by *Alternaria* sp. It causes 5-30% in the entire cabbage growing areas of India (Glory *et al*., 2022) due to *Alternaria* blight's detrimental impacts; the most severe losses can be up to 80% of the yield and 59% of the cabbage seed production can be lost (Hossain and Mian, 2004). Cabbage

leaf blight is caused by two distinct species, *Alternaria brassicicola* and *Alternaria brassicae*. *Alternaria brassicicola* causes dark colored, zonated leaf spots, while *A. brassicae* causes light brown or grey coloured leaf spots on the cruciferous vegetables (Kolte, 1985).

A*lternaria* spots on brassica begin to develop early plantings in seedbeds and due to the disease, the plants become stunted and damped off. Brownish-black discolorations seem on tissue and vary in size, starting with minute spots to larger, *i.e.,* 2 m in diameter. These spots enlarge concentric ring shape and mature abrasion exhibit bull's eye appearance (Siciliano *et al*., 2017). The management of *Alternaria* leaf blight of cabbage is a challenging task due to the presence of multiple inoculums, such as seed, plant debris and cruciferous crops in the soil and the rapid spread of the disease during warm and humid conditions, which can affect a considerable portion of the cabbage harvest. The initial attempts to control it were made using fungicides and cultural practices. However, prolonged and repeated use of fungicides causes environmental and soil pollution as well as residue effects. Hence, integrating or combining the bio-agents and chemicals may be more useful in managing the disease (Sunita and Jha, 2024). Considering the above aspects, the present study aimed to isolate, identify and manage the strategy for leaf blight of cabbage (*Brassica oleracea* var. *capitata* L.) caused by *Alternaria* sp. in foothills of Nagaland.

MATERIALS AND METHODS

Experimental site

The experiment was conducted in randomized block design with four replications at Horticultural Research Farm, School of Agricultural Sciences (SAS), Nagaland University (NU), Medziphema, Nagaland in the field conditions during *Rabi*, 2022-2023. The field is located at an altitude of 304.8 m above the mean sea level and is positioned geographically at latitude of 20° 45' 43" N and a Longitude of 93° 53' 04" E. The initial status of the soil was highly acidic with a pH of 4.4, 1.25% of OC and available NPK of 232.1 kg/ha, 17.81 kg/ha and 209.7 kg/ha respectively (Changkiri *et al*., 2024). During the experimental period, the average maximum and minimum temperatures were 28.8°C and 13.1°C, the average maximum and minimum relative humidity was 91.5°C and 49.3°C, and rainfall was 5.2 mm. The corresponding weather parameters (temperature, relative humidity and rainfall were recorded from ICAR-Research complex for NEH Region, Nagaland Centre, Medziphema, Nagaland.

In vivo study

The experiment comprised of seven treatments, T_1 (seed treatment with consortia of *Trichoderma* sp.*,* TTV -2 + *Bacillus subtilis,* CRB-7 @ 10 g/kg seed and its four periodic sprays @ 1% at 10 days interval started with the initiation of disease), T_2 (seed treatment with consortia of *Trichoderma* sp., TTV-2 + *Bacillus subtilis*, CRB-7 @ 10 g/kg seed + four foliar sprays of copper oxychloride 50% WP @ 0.3% at 10 days interval started with the initiation of disease), T_3 (hot water seed treatment at 52°C for 20 minutes + four foliar sprays of copper oxychloride 50% WP @ 0.3% at 10 days interval started with the initiation of disease), T_4 (hot water seed treatment at 52°C for 20 minutes +four foliar sprays of mancozeb 75% WP @ 0.25% at 10 days interval started with the initiation of disease), T_5 (seed treatment with captan 75% WP @ 3 g/kg + four foliar sprays of copper oxychloride 50% WP @ 0.3% at 10 days interval started with the initiation of disease), T_6 (seed treatment with captan 75% WP @ 3 g/kg + four foliar sprays of azoxystrobin 4.8% + chlorothalonil 40% EC @ 0.25% at 10 days interval started with the initiation of disease) and T_7 (control). The variety 'Pride of India' was used for sowing. The percentage disease index was measured on a 0-5 rating scale as suggested by Conn *et al*., 1990. Where, 0= No visible symptoms, 1= 1-10% leaves infected, 2= 11-25% leaves infected, 3= 26-50% leaves infected, 4= 51-75% leaves infected, 5= Above 75% leaves infected. The percent disease index (PDI) was calculated following a standard formula (McKinney, 1923).

$$\text{PDI} = \frac{\text{Sum of all disease ratings}}{\text{Total number of ratings} \times \text{Maximum grade}} \times 100 \quad \text{Eq. 1}$$

Collection, isolation and identification of the pathogen

Blight symptomatic leaves were collected from Horticultural Research Farm, School of Agricultural Sciences, Medziphema, Nagaland for preliminary microscopic observation. The infected leaves exhibiting typical symptoms were used for isolating the pathogen in potato dextrose agar medium using the standard protocol. Microscopic examination of sporulating pure cultures' morphological and cultural characteristics revealed the fungus. The fungus was determined based on the size, colour, length, width of the culture and number of fungal septations. The isolated pathogen was purified and properly maintained in the laboratory (Adhikary *et al*., 2013).

Pathogenicity test

Susceptible seeds of the crop were surface sterilized with 0.1% $HgCl_2$ before sowing (3 seeds/pot) in pots (25 cm diameter) filled with steam sterilized potting mixture of soil: sand: FYM @ 2:1:1 respectively. One good developing seedling was kept in each pot and kept in a moist chamber to ensure continuous development. The test pathogen was mass replicated on a culture PDA

medium that was placed on petri plates, and the test pathogen's spore-cum mycelial suspension was generated by flooding the plates with 5 to 10 ml of sterile distilled water. The resulting suspension was filtered through double-layered muslin cloth, and the filtrate was diluted with sterile distilled water to obtain an inoculum concentration of 3-5×10^6 spores/ml. Forty-five days old cabbage seedlings was artificially inoculated with an automizer containing spore cum-mycelial suspension. The cabbage cultivated in pots were sprayed with sterile water and maintained as uninoculated control. Both pots were kept in humid environment to further develop leaf spot symptoms in cabbage. The pathogenicity test was carried out, followed by Tu (2015).

***In vitro* study**

The *in vitro* experiment was conducted at the Department of Plant Pathology of SAS, NU, Medziphema, Nagaland. The efficacy was evaluated with two biogents, *Trichoderma* sp. (TTV-2; T_5) and *Bacillus subtilis* (CRB-7; T_6) and four fungicides, *viz*. captan 75% WP @ 0.30% (T_1), mancozeb 75% WP @ 0.30% (T_2), copper oxychloride 50% WP @ 0.25% (T_3) and azoxystrobin @ 0.25% (T_4) were evaluated against the test pathogen by using Dual culture poison food technique (Mohammad *et al*., 2016). Potato dextrose agar (PDA) medium was used as a basal medium. For each concentration, 100 ml of medium was individually weighed for bioagents and fungicides. The weighed quantities for each bioagents and fungicides were then added to a lukewarm PDA at 40-45°C to achieve the desired concentrations.

Efficacy of bioagents

Percent inhibition growth of the test pathogen was calculated using the formula given by Vincent (1947).

$$PI\ \% = \qquad \times 100 \qquad \text{Eq. 2}$$

Where,

PI = Percent inhibition (%) of the pathogen.

C = Average radial growth (mm) of the pathogen in control plates.

T = Average radial growth (mm) of the pathogen in treated plates.

Statistical analysis

The data generated during the present investigation were presented in tabular form and one-way ANOVA at p=0.05 was used to analyse the data as per normal statistical procedures (Panse and Sukhatme, 1985).

RESULTS AND DISCUSSION

Typical symptoms of *Alternaria* leaf spots of cabbage were initially minute; black spots were observed on the older leaves. Then these spots gradually enlarged and became darkened into a circular tan, dark brown to black coloured concentric rings, which gave the appearance of target board symptoms, surrounded by yellow hallows lesions (Fig. 1). As the spots became older, their texture became papery and gave the appearance of shot holes which falls off. The fallen leaves become an inoculum for further disease spread. When the pathogens were isolated and cultured, they were found to be grey olivaceous to greyish black in colour, cottony and zonated mycelia development. The fully developed colony appeared circular and radiating towards the periphery with sporulation on PDA. Microscopic analysis of the test fungus showed that the fungal pathogen *A. brassicae* was grayish olivaceous in colour. The conidia were muriform obclavate with beak of one-third to half the length of conidia, measuring 90-192 µm in length and 4.78-6.71 µm width. The conidia were found to have 7-12 transverse septa, and 0-8 (mostly 0-4) vertical or oblique septa (Fig. 2).

Alternaria brassicicola showed olivaceous grey to greyish green fluffy mycelia growth and irregular margins, obclavate, tapering slightly towards the apex, with the basal cell rounded, beak nearly non-existent, apical cell more or less rectangular or resembled a truncated cone, short thick, sometimes better developed, smooth or slightly warted as age progresses, measuring 30.20-65.34 µm x 21-24.18 µm, with 1-11 but usually less than 6 transverse septa and usually up to 6 longitudinal septa (Fig. 3). The fungal pathogens were identified as *A. brassicicola* (Schw.) Wiltsh and *A. brassicae* (Berk.) Sacc, based on morphological characters. The results obtained were in accordance with reports by Singh *et al.* (2015); Saharan *et al*. (2016); Yadav *et al.* (2016) and Kumar and Shete (2021), who reported on *A. brassicae* from rapseed-mustard for studying physiological, morphological, and cultural variation collected from various geographical regions of India (*viz*., Uttar Pradesh, Uttaranchal, Rajasthan, Haryana, Jammu and Kashmir, West Bengal and Assam). Highest average conidial size (140×20.7 µm and 138.6×22.9 µm). The conidiophore width and length varied from 4.73-6.58 µm and 36.4-36.8 µm respectively. The beak length was 43.35-70.57 µm, and transverse and longitudinal septa varied from 6-8.3 µm and 0.25-2.75 µm respectively. Morphological characteristics of *A. brassicicola* revealed that conidiophores were septate, olivaceous and branched. The conidia were dark cylindrical to ob-

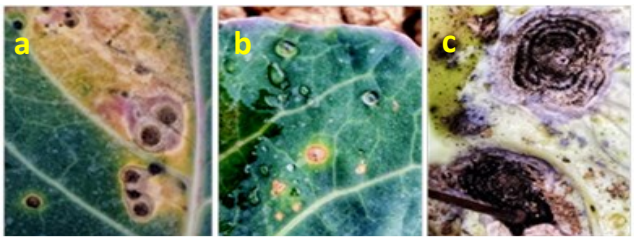

Fig. 1. *Typical symptoms (a-c different stages) of Alternaria* sp. *showing concentric rings*

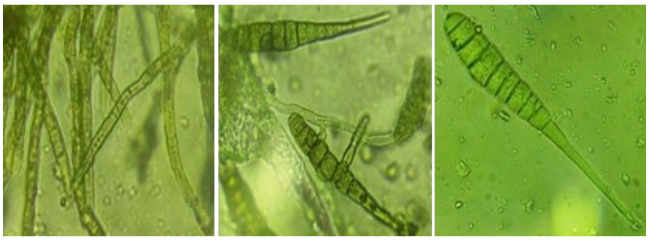

Fig. 2. *Microscopic image of conidia and conidiophores of Alternaria brassicae*

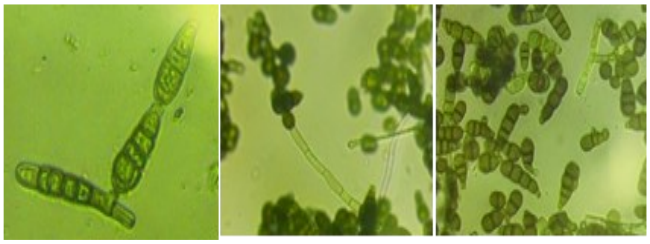

Fig. 3. *Microscopic image of conidiophore and conidia of Alternaria brassicicola*

long, muriform, producing 8-10 spores in chains. There was no prominent beak on the conidia. The conidia had 5-8 transverse and 0-4 longitudinal septations. The sizes of conidia recorded ranged from 13-120 µm in length and 6-16 µm in width (Meena *et al*., 2010; Chand and Chandra, 2014). The pathogenicity testing was observed on a healthy cabbage leaf. The results showed that the first symptoms of the infected plant parts occurred 42 hours after inoculation and additional symptoms occurred, such as small dark brown to black spots that expanded into concentric rings. The fungi were identified as *A. brassicae* and *A. brassicicola*. According to Koch's postulates, the symptoms produced on the leaves and fungal morphology demonstrated pathogenicity. The present investigation aligns with the findings on cabbage leaf blight (*Alternaia brassicae*) by Gunda *et al.* (2018).

The sterilized infected cabbage small leaves pieces were placed on PDA medium in petri dishes. These plates were incubated at room temperature (25±1°C) for proper pathogen development. The treatments differed significantly when compared with control. The disease severity was observed to be significantly reduced. Minimum severity (54.68%) was recorded in treatment T_6 (seed treatment with captan 75% WP @ 3 g/kg + four foliar sprays of azoxystrobin 4.8% + chlorothalonil 40% EC @ 0.25%) followed by treatment, T_5 (seed treatment with captan 75% WP @ 3 g/kg + four foliar sprays of copper oxychloride 50% WP @ 0.3% which recorded disease severity of 64.06% (Table 1). The present findings corroborate with several earlier researchers. Fungicides *viz.,* azoxystrobin used by Mazur *et al*. (2002); Kiran *et al*. (2018), chlorothalonil by Tu and Somasekhara (2015) and azoxystrobin + chlorotha-

Table 1. Effect of Integrated management of *Alternaria* leaf blight of cabbage during *Rabi*, 2022-2023 *in vivo*

Treatment	Alternaria leaf blight (%)	Yield (t/ha)	B:C ratio
T_1: Seed treatment with consortia of *Trichoderma* sp. (TTV-2) + *Bacillus subtillis* (CRB-7) @ 10 g/kg seed and its four periodic sprays @ 1% at 10 days interval started with the initiation of disease	47.53 (43.87)	19.15	2.05
T_2: Seed treatment with consortia of *Trichoderma* sp. (TTV-2) + *Bacillus subtillis* (CRB-7) @ 10 g/kg seed + Four foliar sprays of copper oxychloride 50% WP @ 0.3% at 10 days interval started with the initiation of disease	41.44 (40.36)	21.38	2.40
T_3: Hot water seed treatment at 52°C + Four foliar sprays of copper oxychloride 50% WP @ 0.3% at 10 days interval started with the initiation of disease	49.16 (44.80)	18.22	1.90
T_4: Hot water seed treatment at 52°C + Four foliar sprays of mancozeb 75% WP @ 0.25% at 10 days interval started with the initiation of disease	44.47 (42.11)	20.78	2.31
T_5: Seed treatment with captan 75% WP @ 3 g/kg + Four foliar sprays of copper oxychloride 50% WP @ 0.3% at 10 days interval started with the initiation of disease.	38.20 (38.47)	22.46	2.58
T_6: Seed treatment with captan 75% WP @ 3 g/kg + Four foliar sprays of azoxystrobin 4.8% + chlorothalonil 40% EC @ 0.25% at 10 days interval started with the initiation of disease.	37.69 (38.16)	23.39	2.72
T_7: Control (without treatment)	54.76 (48.02)	17.57	1.80
SEm±	0.60	0.56	
CD @ 5%	1.78	1.66	

Table 2. Percent inhibition of *Alternaria brassicae* at different concentrations till 7th Day (168 hrs) in *in vitro*

Treatments	Per cent inhibition of *A. brassicae* of different hours at 25±1°C							
	24 hrs	**48 hrs**	**72 hrs**	**96 hrs**	**120 hrs**	**144 hrs**	**168 hrs**	**Mean**
T_0: Control (without treatment)	0.36 (3.44)	0.24 (2.81)	0.18 (2.43)	0.12 (1.99)	0.09 (1.72)	0.07 (1.52)	0.06 (1.40)	0.16 (2.18)
T_1:Captan @ 0.30%	61.82 (51.84)	55.95 (48.42)	53.37 (46.93)	61.21 (51.48)	66.38 (54.57)	68.41 (55.80)	72.22 (58.20)	62.76 (52.46)
T_2:Mancozeb@0.30 %	61.82 (51.84)	62.74 (52.39)	58.77 (50.07)	65.29 (53.91)	68.78 (56.05)	71.70 (57.87)	74.44 (59.64)	77.81 (54.54)
T_3:Copper oxychloride @ 0.25%	61.82 (51.84)	44.05 (41.58)	34.19 (35.73)	40.79 (39.68)	44.99 (42.12)	53.93 (47.26)	58.89 (50.12)	48.38 (44.05)
T_4:Azoxystrobin @ 0.25%	61.82 (51.84)	49.17 (44.52)	57.54 (49.34)	65.29 (53.91)	66.34 (54.54)	72.34 (58.28)	76.67 (61.12)	64.17 (53.36)
T_5:*Trichoderma* sp. (TTV-2)	11.82 (19.91)	23.69 (29.08)	16.65 (18.77)	48.95 (44.40)	54.82 (47.77)	63.80 (53.02)	69.44 (56.45)	41.31 (38.49)
T_6:*Bacillus subtillis* (CRB-7)	14.09 (21.84)	10.12 (18.32)	4.22 (10.84)	9.17 (17.30)	13.88 (21.80)	25.63 (30.40)	31.11 (33.90)	15.46 (20.06)
SEm ±	1.11	1.08	3.85	1.01	0.81	0.48	0.40	
CD @ 5%	3.26	3.17	11.33	2.98	2.37	1.40	1.17	

Mean of four replication and those in parentheses are arcsine transformed values

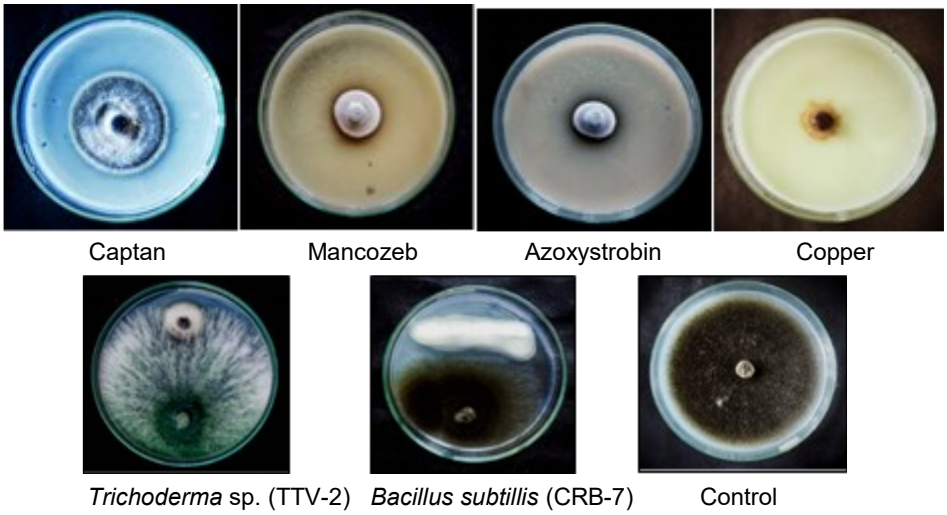

Fig. 4. *Efficacy of fungicides and biocontrol agents on Alternaria brassicae*

lonil by Ekabote *et al*. (2019) were effective against *Alternaria* leaf blight. Azoxystrobin, under the strobilurins group of fungicides acted as mitochondrial electron transport inhibitors.

Two bio-control agents, namely *Trichoderma* sp. (TTV-2; T_5) and *Bacillus subtilis* (CRB-7; T_6) were tested using a dual culture technique to determine their antagonistic effect against *A. brassicae* under *in vitro* conditions. Among the two bio-agents, *Trichoderma* sp. (TTV-2; T_5) showed a significant antagonistic effect, with 69.44% inhibition and confined the pathogen growth to 42 mm on 7th day of incubation. The bacterial antagonist gave 31.11% inhibition with pathogen growth to 62 mm (Table 2, Fig. 4). A study by Verma *et al*. (2006) found that the bioagents showed fungistatic activity against *A. solani* and significantly decreased the growth of the strain over control. Among all tested bioagents, *T. harzianum* significantly inhibited the growth of *A. solani* with 65.88% inhibition, followed by *T. viride* and *P. fluorescens* which showed 48.23% and 38.82% inhibition, respectively. Marchande *et al*. (2020) observed similar effects to bacterial (*Pseudomonas fluorescens*) and fungal (*T. virens*, *T. harzianum* and *T. koningii*) bioagents against *A. alternata* in *in vitro* conditions.

In the poisoned food technique, azoxystrobin (T_4) yielded 76.67% inhibition of *A. brassicae* on the 7th day of inoculation, followed by mancozeb and captan with 74.44% and 72.22% inhibition, respectively. Copper

oxychloride showed least inhibition with 58.89% (Table 2, Fig. 4). Among the six fungicides *viz.,* captan, mancozeb, carbendazim, azoxystrobin and chlorothalonil were evaluated under *in vitro* conditions, azoxystrobin was the most effective with 90.42% of mycelial inhibition (Waghe *et al*., 2015; Patel *et al*., 2018). The results obtained on the fresh yield of cabbage revealed that the highest yield (23.39 t/ha) was obtained from T_6 followed by T_5 (22.46) was calculated for the yield supplemented by applying different practices to manage *Alternaria* leaf blight of cabbage. The Cost-benefit ratio (CBR) data showed that the highest ratio was obtained from T_6 (2.72), followed by T_5 (2.58).

Conclusion

The present study concluded that *Alternaria* leaf spot of cabbage caused by *A. brassicae* and *A. brassicicola* was potentially devastating. In the poison food technique, Azoxystrobin treatment was most effective and showed the highest disease reduction percent. The integrated approach with an effective fungicide (azoxystrobin) coupled with bio-control agent (*T. harzianum*) could effectively reduce the severity of *Alternaria* leaf spot of cabbage. Integrated management method is one of the holistic strategies for managing various diseases with the application of proper doses and at the right time, bringing maximum reduction in disease incidence. However, more extensive research, focusing on eco-friendly management, such as the 'Integrated Disease Management' method, must be experimented with for its confirmation to deal with cabbage leaf spot found to be highly damaging to the crop. It is also important to identify and isolate more potential resident biocontrol agents. Further research is required to produce eco-friendly chemicals, etc., for effective management of cabbage leaf spot diseases.

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Conflict of interest

The authors declare that they have no conflict of interest.

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