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FIELD STUDY ON INTEGRATED DISEASE MANAGEMENT OF CABBAGE HEAD ROT CAUSED SCLEROTINIA SCLEROTIORUM

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A B S T R A C T

Cabbage head rot caused by fungi Sclerotinia sclerotiorum is a very common problem for vegetable growing farmers. Diseased stem and leaves produce white cottony growth and hard sclerotia. Cabbage is used as fresh green salads thus disease management is not safe and economical by using solely chemical therapy. An integrated approach was designed in 4 years trial evaluation to minimize disease incidence. In first step best treatments were selected by using methods of in-vitro food poison evaluation method, cultural management, soil fumigation and foliar treatments by botanical extracts and modern fungicides. Cultural practices included north south row orientation, crop sanitation by elevated ridges, and humidity management by adjusting irrigation schedule showed control of up to 15%. Soil fumigation by Thiophenate Methyle and score reduced disease incidence by 50.33%. Among the five bio-extracts foliar application of jatropha extract showed best control of 31%. From four fungicides Amistar Top showed highest control of 73% in 2018 and Score produced best results of 76% in 2019. In second step best treatments were combined in a single application integrated strategy which reduced incidence by 80.5%. Farmers were advised to adopt safe methods keeping in mind the extant of disease incidence and crop loss.

Keywords: Integrated Disease Management, Cabbage, Cultural control, *Sclerotinia sclerotiorum*

INTRODUCTION

Cabbage (*Brassica oleracea L. var capiata*) of brassica family, is economically important agricultural crop which is grown throughout the world (Singh *et al.*, 2010). It is highly nutritious vegetable, used in animal fodder sauerkraut, slaw and most often in food dishes (Fang *et al.*, 2005). It improves digestive process in stomach and reduces constipation and cancer incidence Jiao *et al.* (2010). In Pakistan it is grown as Rabi vegetable and has become a leading vegetable which is cooked in dishes and used extensively in salads. In recent years its cultivation has increased from 3000 ha to 5000 ha (Govt. of Pakistan, 2016). Its average house hold consumption is 3.5kg per month in Pakistan (Fayaz *et al.*, 2014) but its yield is not as high as in other cabbage countries specially in case of seed crop due to attack of diseases and pests. The most common quality and yield limiting factor in cabbage is its head rot disease caused by Sclerotinia sclerotiorum (Loganathan *et al.*, 2010) It was first reported in: New York (Dillard and Hunter, 1986), North Carolina (Cubeta *et al.*, 1997), in Texas (French and Schultz, 2009) and New Zeland(Jones *et al.*, 2014). In Mexico, Sanogo *et al.* (2015) isolated *S. sclerotiorum* from grown rotted cabbage leaves and confirmed it through using PCR technique. Mahalingam *et al.* (2016) found *S. sclerotiorum* causing head rot of cabbage during a survey in Sri Lanka and verified the pathogen through PCR amplification of its rDNA region using ITS1 and ITS primers. *S. sclerotiorum* as the pathogen causing head rot of cabbage was confirmed for the first time in Pakistan by (Mirza and Ahmad, 1988) from the cabbage samples found from field of NARC Islamabad.

The fungus *S. sclerotiorum* is a nonspecific plant pathogen with a broad host range (Saharan and Mehta,

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2008), infects more than 400 plant species (Boland and Hall 1994). It produces only sexual ascospore but no asexual spore. Its pathogenicity factors involve enzymes that degrades cell wall and Oxalic Acid, mycelia virulence decreases with its age (Wang *et al.*, 2016). In cabbage it causes severe losses to crops in field, to produce in storage, transport and market processes (Dillard and Hunter, 1986). It can survive long time in soil as sclerotia (Adams and Ayers 1979; Willetts and Wong 1980), in favorable conditions its sporulation attack the leaves in contact with soil (McLean, 1958). Early symptoms include white spots on stalk , a soft rot develops with characteristic symptom of luxuriant white cottony mycelium with enlargement of water soaked lesions affecting entire head or leaf layers and black hard and large sclerotia forms in the head (Sherf and MacNab, 1986). The whole plant wilts and topples by collapsing stem near the soil, pathogen invades pith to cause death prior to production of seeds (Hoes and Huang, 1975). The fungus grows and transmits to other crop plants causing severe damage to yield and quality of cabbage crop (French and Schultz, 2009).

Control of head rot of cabbage has remained a serious concern in Pakistan for large and medium field farmers. No variety of *Brassica oleracea L. var capiata* yet provides significant resistance against *S. sclerotiorum* (Dickson and Petzoldt, 1994; Hudyncia *et al.*, 2000; Mahalingam *et al.*, 2017). For this soil born fungus, cultural practices such as weed eradication and crop rotation did not served the purpose for complete control because of pathogen's wide host range coupled with its ability to survive in the soil for long times (Adams and Ayers 1979; Willetts and Wong 1980). *S. sclerotiorum* can survive as sclerotia in soil up to ten years thus one year of crop rotation is not enough to survive pathogen attack when conditions become favorable. It's control through cultural practices is not sufficient and effective in favorable conditions (Young and Werner, 2012). Biological control of S.sclerotiorum of cabbage with trichoderma, chitin based (Loganathan *et al.*, 2010) and Coniothyrium minitans with Trichoderma spp. has shown some potential on experimental basis pots in lab (Jones *et al.*, 2014). But these products require mass multiplication for field application and are not easily available yet in market as commercial products (Loganathan *et al.*, 2010); thus there practice in field is not adapted and neither there application proved economical for commercial cabbage growers.

Disease caused by *S. sclerotiorum* is not managed consistently and economically in field due to their ability to with stand the adverse environmental conditions and sporulation on arrival of favorable environmental conditions. Rather than relying on single control strategy combined measures have to be applied for management. (Mila *et al.*, 2003). It has become very well understood fact now different methods must be combined for maximum control of *S. sclerotiorum* disease into a single management program. (Saharan and Mehta, 2008). Integrated disease management (IDM) is a technique of combining range of best possible measures and involves the use of all disease management approaches practically available to farmers which can include cultural, biological and chemical methods with aim of keeping disease incidence lower than economic threshold levels.

Cultural practices can be modified to maintain low level of humidity to minimize leaf injury in cabbage field as duration of humidity is an important factor for disease development by *S. sclerotiorum* (Tores and Moreno, 1991). Dry weather conditions reduce cabbage white mold development caused by *S. sclerotiorum (Dillard et al., 2004).* Flooding schedule in which a field is continuously flooded in 23–45 days cycles of alternate flooding and drying led to destruction of sclerotia of *S. sclerotiorum* and reduced disease in Florida and India (Singh and Tripathi, 1996).

Soil chemical treatment is very important to reduce pathogen pressure. If left untreated *S. sclerotiorum* can survive in soil in form of sclerotia for ten years which reduces the effectiveness of technique of crop rotation for management of white mold (Young and Werner, 2012). Alabouvette and Louvet (1973) effectively used methye bromide and formaldehyde in pre-plant treatments to destroy sclerotia in the soil. Sclerotinia can be controlled effectively by soil fumigation in tobacco by Benomyl and thiophanate-methyle (Hartill and Campbell, 1973). Carbendazim controlled sclerotinia rot in peas when applied in the soil before sowing of pea s (Sugha, 1999).

Chemical control is used only when it is necessary when disease incidence climbs to economic threshold levels in the way so it can only cause least disruption for natural mechanisms involve in vegetable consumers, harvesting and handling personal and environment because it is harvested in several pickings by manual method. IDM technique, do not eliminate chemical therapy for control but it allows judicious use of fungicides by safe and wise decision making principles. Razdan and Sabitha (2009)

Fungicides are routinely applied to eradicate fungal spores, reduce its germination potential, to check its mycelium development and to break its disease cycle (Turkington and Morrall, 1993). But no fungicide has yet been registered specifically for control of white mold or head rot of cabbage. Lack of a registered fungicide, non-availability of completely resistant varieties and sporadic occurrence of this disease on large commercial farms has made its management very difficult (Hudyncia *et al.*, 2000). Thus there is a dire need to identify relatively safe chemicals for its control. Hence the present study was focused to evaluate most suitable cultural methods, soil treatments along with modern foliar fungicides that can be safely used against *S. sclerotiorum* for complete control of cabbage head rot.

MATERIAL AND METHODS

Field experimental trial was conducted in Rabi 2017-18, Rabi 2018-19 and Rabi 2019-20 in the field of Plant Pathology Research Institute where 85% cabbage mortality was recorded previous year. Purpose of trial was to devise an integrated approach for farmers and to evaluate the suitable cultural practices, efficacy of plant extracts and fungicides for lowering disease incidence and yield losses. Seed of variety "Golden Acre" in Punjab was obtained from the Vegetable research institute, Faisalabad. Four separate trials were conducted for in-vitro, Cultural, Bio-extract and Fungicidal management of cabbage head rot. Cabbage seeds for nursery plants were sown in 2^{nd} week of October and plants were transplanted in 1^{st} week of November in open field in rows with planting distance 60cm between rows and 40cm within plants as described by Jensen *et al.* (2005). Soil type was sandy loam and after thinning number of plants retained in each row was 20 Insect control and fertilizer applications were carried out in field according to local practices. Urea, 46% was applied thrice during growing season at a rate of 100 kg ha^{-1} in experimental field. To control insects Acelam 26% WDG (Acetamiprid 23.5% + Lambda Cyhalothrin 2.5%) at rate of 60 g ha^{-1} was sprayed before inoculating crop with fungus. Trial was laid out in Randomized Complete Block Design with 3 replications having area of 14m x 9m each.

Isolation and multiplication: Diseased samples of cabbage head rot were collected from different farmer fields and were brought to lab. Leaves with prominent symptoms were cut into 5mm small pieces, surface sterilized with 0.5% solution of sodium hypochlorite. These were placed in 90mm petri plates already poured with PDA media and dishes were placed in temperature of 25°C. After 3 days fungal colony of *S. sclerotiorum* was visible with white mycelium and culture was transferred to another PDA plate for purification, where black hard structures surrounded with crystal shaped clestothecia started to develop on white fungal colonies which became sclerotia of 2mm with irregular shape.

In vitro Evaluation of Fungicides: For in vitro efficacy of fungicides Potato Dextrose Agar media was prepared by adding four fungicides at 0.001 PPM concentration at 45°C, media was poured in Petri dishes and 5mm inoculum disc of *S. sclerotiorum* culture was placed in center according to method of Dalili *et al.* (2015) with three replications. Plates were incubated at 22°C and results were noted after four days for fungal growth inhibition by fungicides according to Pandey *et al.* (1982).

Field Multiplication of inoculum: For the purpose of mass multiplication in field 2kg chickpea were boiled for 30 minutes on 90°C and then dried for 20 minutes by spreading on paper. Transparent plastic bags were filled with 0.2kg of grams and were sealed with cotton by placing cork plug; these bags were autoclaved at 121°C to kill all micro-organisms. Fungal culture of 6mm size were inoculated in those bags and were place on 30° for 10 days, whitish cottony fungal colony was fully developed on grams. These were mashed and spread in each replication in field near stems of three week old crop plants selecting a day with light precipitation of rain. Field was irrigated on alternate days for two weeks to promote growth and infection of fungus, on every evening water was also misted on all floral parts of plants to maintain moist conditions, cool wet weather in fall supports head rot development in cabbage (Hudyncia *et al.*, 2000). Symptoms started to develop on 5^{th} day of inoculation on lower stem in form of cottony white mycelium formation.

Cultural Practices: Cultural practices such as moisture regulation and field sanitation can be modified to reduce moistness and extend dry period in cabbage grown fields. Thus effect of height of furrows and Time of irrigation was evaluated. Furrows were made north and south facing as reported by Bennett and Elliot (1972) incidence and severity of sclerotinia damage can be reduced by adopting north and south facing row orientation. *DI%* was compared for furrow height of 7 inches with 5 inches and *DI%* of morning irrigation time with evening irrigation time. First treatment was 7" furrow with evening irrigation, second treatment was 5" furrow with Evening irrigation, and third treatment was 5" furrow with Morning irrigation, while fourth treatment was 7" with morning irrigation. Disease incidence was calculated by *DI*% formula.

Soil Treatment: Thus field area was flooded by adding chemicals in irrigation water before transplanting nursery to destroy fungal sclerotia. Five chemicals tested were Carbandazim 500gm/ha, Topsin M 500gm/ha, Score 750ml/ha, Alliete 1Kg/ha, Topsin M 500gm/ha + Score 750ml/ha. And no chemical was added in control treatment. *DI%* was calculated each year by formula after comparing incidence in treated with untreated control.

Foliar Application of Chemicals and Plant Extracts: In cabbage crop cultural practices prevents disease development to some extent but most effective method found for control of S. sclerotiorum is chemical application (Mahalingam *et al.*, 2017). Five chemical treatments viz Nativo 75%WG (Tebuconazole + Trifloxystrobin) @ 0.50gm/liter, Score 250EC (Difenoconazole) @ 0.125ml/liter, Kocide 3000 52.4%WG (Copper Hydroxide) @ 0.25gm/liter, Amistar Top 325SC (Azoxystrobin + Difenoconazole) @ 0.30 gm/liter were sprayed on foliar parts by using manually operated knapsack sprayer. First foliar spray was applied on the onset of disease and was repeated after two weeks.

Five plant extracts Tovacco, Neem, Clove, Jatropha and Garlic were used for the control of *S. Sclerotiorum*. Disease incidence and inhibition was calculated each year by formulas after comparing *DI%* in treated with untreated control rows. While in control treatment plants were sprayed with water only.

Combining best treatments for IDM approach: Three treatments with replication were used in 2020 trial by combining best control treatments from previous years to evaluate integrated disease management strategy for Cabbage Head Rot. First combination was flooding field with Score 750ml/ha + Topsin M 500gm/ha field with irrigated in morning, inoculum was spread in field by infected grams method, on appearance of first symptom Score was sprayed on foliar parts @0.125 ml/lit of water. In second treatment Jatropha extract was sprayed on foliar parts with all other combination of first treatment. In third treatment no chemical was flooded neither was applied on foliar part with morning time irrigation. DI% was calculated by using formula and disease reduction was compared with untreated control.

Data Recording: There were five treatments and each treatment had 80 plants in each replication and 240 plants in 3 treatments. All the chemical treatments were sprayed twice in Randomized Completely Block Design (RCBD). Head rot Incidence was expressed by percent disease index (PI) was calculated after heading stage (Loganathan *et al.*, 2010) by using formula:

$$\text{Disease incidence (\%)} = \frac{\text{No. of infected plants}}{\text{Total no. of plants}} \times 10$$

Disease reduction over control was calculated after final spray by using the formula;

$$\text{Disease reduction over control (\%)} = \frac{\text{Control} - \text{Treated}}{\text{Control}} \times 100$$

RESULTS AND DISCUSSION

The results were analysed statistically by analysis of variance and means were separated using least significant differences (L.S.D) test by using Statistix 8.1 software.

In vitro Evaluation of Fungicides: Efficacy of four fungicides at viz. Nativo, Score, Kocide, Amistar Top was checked by food poison method while comparing means of mycelial growth in chemical added PDA plates to means in simple untreated PDA plates. All fungicides showed significant response in control fungal growth. Amistar Top and score shared same levels of significance while Amistar Top showed maximum fungal growth inhibition 98.48% whereas Kocide showed minimum growth inhibition 80.23%. Table shows means of mycelial growth in PDA made with fungicides.

Table 1. Mycelial growth(MMG) of Sclerotinia Sclerotiorum and inhibition % in petri plates by food poison method.

Food Poison Method 2017		
	MMG (mm)	Growth inhibition (%)
Nativo	4.67 C	94.68
Score	3.00 CD	96.58
Kocide	17.33 B	80.23
Amistar Top	1.33 D	98.48
Control	87.67 A	

Cultural Practices: Cultural practices can play important role in controlling disease losses thus *DI* (%) was checked while keeping the crop untreated by any soil and foliar chemical. DI (%) was greater at 5” furrows, cabbage leaves got more in contact with ground and irrigation water resulting caused leaf injuries that increased ingression of *S. Sclerotiorum Dillard and Cobb (1995)*. DI% differed significantly in morning and evening irrigated fields. Morning irrigated field showed less DI (%) when compared with evening irrigated crop due to extended moist period was extended in the evening irrigation, as infection process is favored by cool and moist conditions of leaf wetness for 16 hours (French and Schultz, 2009). Table shows DI (%) on different furrow height and irrigation during the year of 2018 and 2019. Results showed that timing of irrigation is more important than height of furrows.

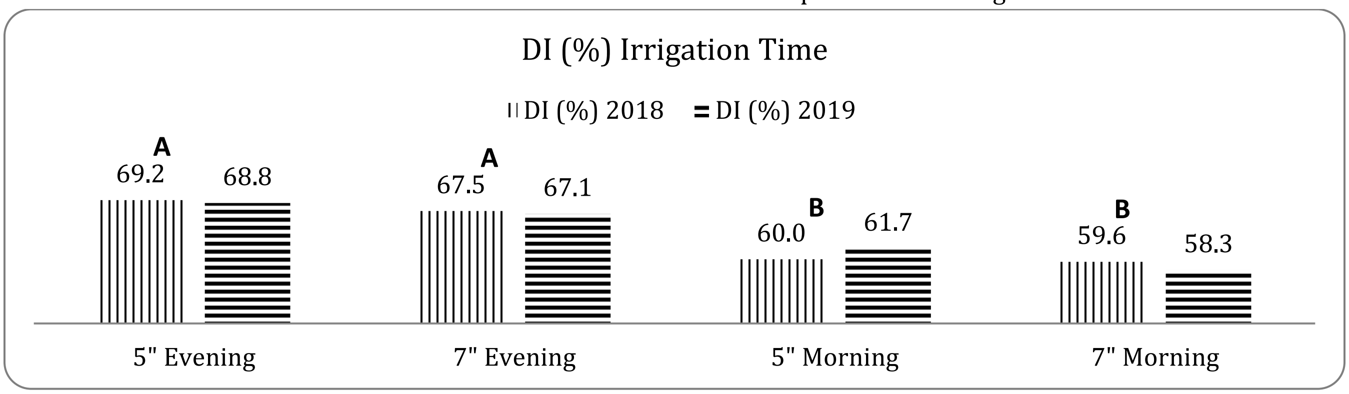

Figure 1. Bar diagram shows DI% for morning and evening irrigated crops. Vertical Pattern shows DI% for the year of 2018 while Horizontal Pattern shows data for the year of 2019.

Soil chemical Treatment: Six soil treatments were tested in isolation for control of cabbage White Mold without applying any foliar application. Score + Topsin M showed maximum disease reduction of 51% for both years when compared with control treatment. Fungicides inhibit the sporulation of fungal sclerotia present in soil thus lower the disease incidence by decreasing soil inoculum pressure.

Table 2. Disease incidence % and disease reduction % by soil chemical treatment method for the year of 2018 and 2019.

Treatment	2018		2019	
	DI (%)	Decrease Reduction (%)	DI (%)	Decrease Reduction (%)
Score + Topsin M	29.58 E	51.70	31.25 D	50.33
Score	33.75 D	44.90	35 CD	44.37
Carbendazim	39.58 BC	35.37	43.33 B	31.13
Topsin M	36.25 CD	40.82	36.67 CD	41.72
Aliette	40 B	34.69	38.33 BC	39.07
Control	61.25 A		62.92 A	

Foliar Application of Chemicals and Bio-extracts: From four chemical treatments Amistar Top showed maximum disease reduction in 2018, while Score showed maximum disease reduction in 2019. Among chemicals minimum control was shown by Kocid of 50% and 57% in 2018 and 2019 respectively. From the Bio-extracts Jatropha extract showed maximum disease control of 32% and 30% in 2018 and 2019 respectively. When mean values of chemical treatments were compared with control by applying LSD test, all treatments showed significant response while Amistar Top and Score showed same level of significance among foliar chemicals. From bio-extracts, Jatropha showed maximum disease control of 32%, while tobacco extract showed minimum control of 14% and 10% and 2018 and 2019 respectively.

Table 3. Disease incidence% and control% for chemical and bio-extracts for the year of 2018 and 2019. Treatments sharing same letters have same level of significance.

Foliar Treatment Comparison				
Treatment	2018		2019	
	DI (%)	Control (%)	DI (%)	Control (%)
Kocide	31.25 F	50	27.5 F	57.42
Nativo	25 G	60	23.33 G	63.87
Score	17.08 H	72	15 H	76.77
Amistar Top	16.6 H	73.44	15.42 H	76.13
Tobacco	57.92 B	14.20	57.92 B	10.32
Garlic	55.83 BC	17.28	54.58 BC	15.48
Clove	52.92 CD	21.60	51.67 CD	20
Neem	50.83 D	24.69	49.58 D	23.23
Jatropha	45.83 E	32.10	45 E	30.32
Control	67.5 A		64.58 A	

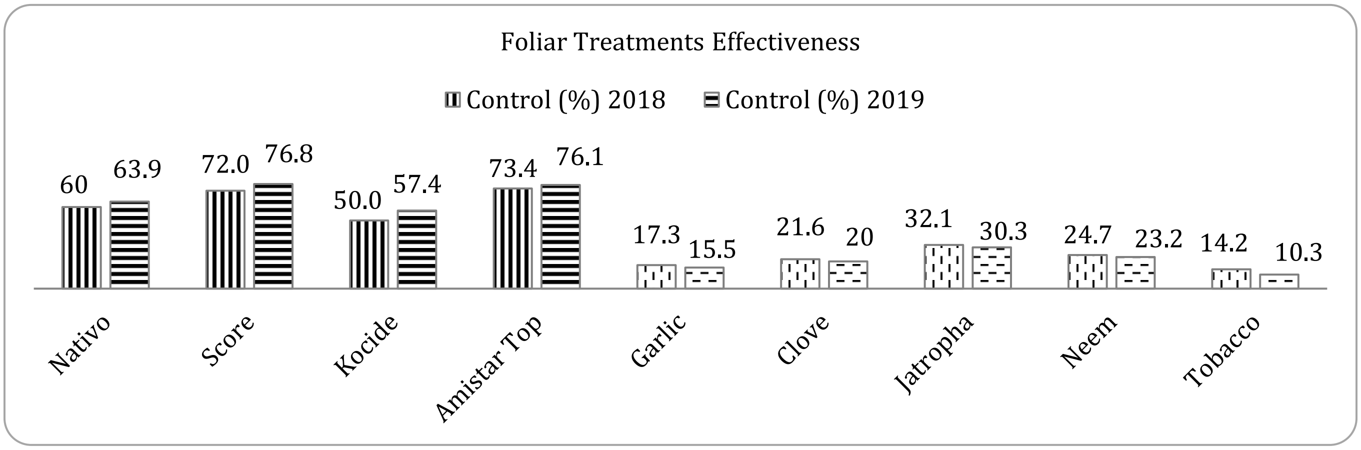

Figure 2. Bar diagram shows disease reduction % of fungicides in line pattern and bio-extracts in dotted pattern. Vertical patterns show data of 2018 and horizontal shows data of 2019.

Combining Treatments for IDM: Treatments shown good results in previous years were applied in single combination and Disease Reduction is mentioned in table 5.

Table 4. DI% and effectiveness of treatments applied in combination for Integrated Disease Management in Rabi 2019-20.

Treatment	Disease Incidence (%)	Reduction in Disease incidence (%)
T1 (Morning Irri + Score + Flood Score + TopsinM	12.08 C	80.54
T2 (Morning Irri + Jatropha + Flooding Score + Topsin)	19.58 B	68.46
Control	60.08 A	

In T1 soil was treated with Score + Topsin M, crop was weekly irrigated on morning, score was applied as foliar fungicide.

In T2 soil was treated with Score + Topsin M, crop was irrigated in morning, Jatropha extract was applied as foliar application. T1 showed 80% control and T2 showed 68% control.

CONCLUSION

Single strategy management approach for control of diseases found in largely sown crops was less efficient and less economical. Rapidly emerging phenomenon of pathogenic resistance and rapidly changing environmental conditions in Pakistan have made it necessary to adapt techniques which combine a variety of control measures in single strategy. Although disease management by applying foliar chemicals is very popular and reliable approach, but chemicals pose environmental hazards residual effect of chemicals is most important concern in vegetables especially in salads and leafy vegetables like cabbage in which green leaves are consumed directly without further boiling and cocking. Thus finding a safe chemical to control head rot was biggest challenge along with its efficacy. Hou *et al.* (2016) studied residual effect of azoxystrobin in cabbage under field conditions and found that it is safe for cabbage disease control as it was dissipated rapidly with the mean half-live of 0.4 days at three geographical experiments, its terminal residues in cabbage at harvest time were below the maximum residue limit (MRL, 5mg kg^{-1} for azoxystrobin) established by Codex Alimentarius Commision.

Difenoconazole is another systemic fungicide with IUPAC name: 1-(2-[4-(4-chlorophenoxy)-2-chlorphenyl]-4-methyl-1, 3-dioxolan-2-yl methyl]-1 H-1,2,4-triazole, it is very effective to control white mold caused by Sclerotinia sclerotiorum (Pandey *et al.*, 2012).

Pathogens evolve resistance against fungicides when they are applied frequently and non-judiciously, improper fungicidal applications lead to development of fungicide resistant isolates. Mahalingam *et al.* (2017) found sclerotinia sclerotiorum isolates from cabbage that were resistant against Tubeconazole (Nativo) thus reducing its efficacy against head rot disease. Frequent chemical application leads to development of pathogenic resistance thus integrated disease management approach was adopted to combine best suitable treatments for the control of cabbage head rot disease. Management practices for disease limiting factors should be adopted such as weed control, day timing and frequency of irrigation, sowing of disease free nursery should also be given proper importance along with chemical control.

There is need to develop resistant varieties of cabbage which can show field resistance against sclerotinia sclerotiorum and a disease predictive model should also be developed by studying important epidemiological factors.

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Muhammad U. Yasin	:	Conduct research and wrote manuscript
Saba Saeed	:	Provide technical assistance in conducting research
Saima Naseer	:	Review manuscript