

Powdery Mildew A Disease of Grapes And The Fungicides Mode of Action: A Review

Mueen Uddin¹, Juma Khan Tareen², Faheem Ahmed², Faisal Adnan², Mohammad Jafar Bazai², Syed Rehan Fareed², Hidayatullah Kakar^{2*}

¹Department of Plant Pathology, Lasbela University of Agriculture, Water and Marine Sciences, Uthal Lasbela, Balochistan, Pakistan

²Agriculture Research Institute (ARI), Sariat Quetta, Balochistan-Pakistan

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Abstract: Powdery mildew caused by *Uncinula necator* (Schw.) Burr. is one of the widespread fungal diseases of grapes that have caused economic losses through poor fruit set and low yield substantially. To decrease the inoculum potential, a disease management program must be undertaken early in the season which is imperative to reduce late-season disease problems. Because, without early control of the infection of powdery mildew, often lead to severe problem in the late season. Elemental sulfur was the foremost antifungal utilized for the control of powdery mildew which is still in use as an effective and cheap fungicide for vineyards. Sterolbiosynthesis inhibitors (SI), also called SI fungicides, are the latest products to control powdery mildew effectively. For efficient use of fungicides with no or less resistance to the pathogen, it is appropriate to spray fungicides having different mechanisms of action which are specific in function, and for more efficacy, use a mixture of such fungicides that have no harmful impact on plant growth and environment. So, for effective control of powdery mildew, a protective spray of fungicide before bloom and a subsequent spray of systemic fungicides at the time of berry formation ensure healthy and higher grapes yield. This paper reviewed the powdery mildew infection in grapevines and its effect on yield losses with details of fungicides' mechanism of action and newly developed fungicides having broad spectrum activities

Keywords: Economic losses, fungal diseases, grapes, fungicides, mechanism of action,

***Correspondence:** Hidayatullah Kakar, Agriculture Research Institute (ARI), Sariat Quetta, Balochistan, Pakistan, Tel: 0092- 21-34650726 Email: kaintkk@gmail.com

Introduction

Viticulture is practiced in temperate and subtropical agroecological zones globally. Grapes (*Vitis vinifera*) have their place in Vitaceae and are successfully grown in diversified climates and soil as compared to other deciduous fruits. Sandy loam soil which has low water holding capacity is most conducive to its growth. In Pakistan, Balochistan is dominant in grape production and contributes 67% to the production of the national grapes followed by KPK (Khyber Pakhtunkhwa) producing 1.0 thousand tons annually. Grapes' potential yield is 25 tons ha⁻¹ but the growers are hardly obtaining an average yield of 5.92 tons ha⁻¹ [1].

In the uplands of Balochistan, different local and exotic varieties of grapes are grown in Quetta, Pishin, Killa Abdullah, Mastung, Kalat, Loralai, and Zhob districts. The most popular commercial varieties grown in these districts include Haita, Kishmishi, Shundokhani, Sahibi, Shekhali, and Red Goble [2]. Similar to other deciduous fruits, the annual life cycle of grapes is comprised of six stages including i) bud break, ii) vegetative growth, iii) blooming, iv) fruiting, v) ripening and vi) shedding of leaves. In the autumn season grapes shed leaves and then endure a dormant period from December to the end of March in winter [3]. Due to these

growth stages, only 100 days become available from the berry setting to the harvesting of the grapevine.

The disease-susceptible Grapevine when planted in areas having high pathogen stress cause greater economic loss. In such a situation, the use of a fungicide is the only option to control the disease [4]. Generally, fungicides are used at a stipulated time to get the plant surface covered as a preventive tool. Sometimes, a number of fungicides are repeatedly sprayed with respect to the time in the production cycle of a crop. The efficacy of fungicide is decreased when sprayed too early or late when the disease is in epidemic form [5]. Powdery mildew (*Erysiphe necator*) affects grapes tremendously and non-commercial varieties have less resistance against this pathogen that requires 6 or more fungicide sprays for effective control [6,7]. It has been reported that one week before anthesis bunches with berries size of 2 mm is a highly susceptible stage to disease attack [8]. This paper reviews the powdery mildew infection in grapevines and its effect on yield losses with details of fungicides' mechanism of action and newly developed fungicides having broad spectrum activities.

Powdery mildew

A dry climate is quite conducive to the development of powdery mildew but it can also be spread over a wide range of humidity levels [9]. The majority of grapes cultivars grown in Asia are susceptible to fungal diseases particularly powdery mildew including *Vitis amurensis*, *V. armata*, *V. davidii*, and *V. romaneti* while, the less susceptible grape cultivars grown in the United State of America are included *V. aestivalis*, *V. berlanderi*, *V. candicans*, *V. labrusca*, *V. riparia* and *V. rupestris* [10]. The infection caused by *Unicula necator* in grapes susceptible cultivars produces inferior quality fruit fetching less market price along with a severe reduction in yield and growth of vine as well as a decrease in hardiness during winter.

The characteristic features of fungal infection by *Ucinula necator* on grapes are manifested in the form white dusty appearance on the leaf surface which is the conidia of *U. necator* develops from its mycelia. The cleistothecia of the fungus overwinter on the bark of vine, when getting moisture onset of the spring season, become stimulated and release ascospore which reaches to the infection site through water splash or through air blown. The first symptoms appear as chlorotic spots on the leaf's upper surface of small size, sometimes as shiny spots or a white web on the lower side of the leaf. While, on the shaded leaves, both sides show similar symptoms of grapevine. Those leaves or shoots which are close to the bark indicate the primary symptoms of infection [9]. It has been noted in grapevines that high infection of powdery mildew in the preceding time also showed dusty or powdery coverage of buds by the hyphae of *U. necator* in the current season which overwinters on the inner bud scales. At the time of bud break, the cleistothecia are stimulated and release various conidia and mycelium ready to cause infection on new shoots that appear light color which is the flag leaf [10].

Once the primary infection caused by the spread of conidia at the start of the spring season triggers the secondary infection of *U. necator* through the wide spread of conidia by water and/or wind blowing to other parts of the grapevine after bud break. The mechanism of infection is initiated through penetration of haustoria into the epidermal cells of the infected site and shows symptoms of necrotic [10]. Other symptoms appear in the form of distorted or stunted growth of young newly emerging leaves while the petioles become brittle and break after infection by *U. necator* [9]. Further, the powdery mildew spread to green shoots and exhibits red and brown lesions, and around bloom, the grapes bunches get infected leading to a decrease in fruit set as well as yield of grapes. When the haustoria of conidia penetrate into the epidermal cells of berries results in the killing of berries or the berries' skin show scarring and spitting. The infected grapes become unmarketable due to off-flavor and lack of freshness [9]. The infected berries with split skin become further vulnerable to other diseases predominantly fruit rotting (*Botrytis cinerea* Pers.) and the early infection often produced stunted berries [10].

A team of researchers investigated the wine quality as affected by powdery mildew and observed that infected berries used for winemaking resulted in reduced acidity with more total soluble solids levels when compared to wine prepared from healthy berries. They further noted that wine prepared from infected grapes also showed faster browning while such wine also indicates off-flavor when tested through sensory evaluations. According to a group of researchers that only 3% of infection of berries by *U. necator* produced off-flavor in wine and also increased acid levels in berries. In addition to that increased acid concentration in fruit can spoil the quality of the wine [11, 12].

The yield losses of grapes occur due to infection of powdery mildew (*U. necator*), one reason is the poor fruit setting immediately after infection and long-term losses happen due to a decrease in growth and hardiness during winter [10]. Another reason for the low yield of grapes is the reduction in photosynthesis and water use efficiency under severe powdery mildew infection [13]. A group of researchers in New York evaluated grapevine cultivars against powdery mildew and noted that the susceptible cultivar (White Riesling) revealed a reduction in photosynthesis rate due to deterioration of the leaf palisade cell layer as compared to the less susceptible cultivar (Concord leaves) where both cultivars were subjected to the infection of powdery mildew [13]. It further reduces the leaf size which might be resulted in a decrease in the photosynthesis rate of the grapevine [14]. Many of the infected buds were killed during cold winter which led to yield losses in grapevines grown in cold agroecological conditions.

Life cycle of *Uncinula necator*

The *Uncinula necator* sustains its life cycle by adapting two strategies as overwintering [Fig 1]. In agroecological conditions where winters are relatively mild, the mycelium of *U. necator* overwinters in dormant buds on primordia leaf. Subsequently when the spring season onset the mycelium becomes stimulated and starts causing a severe infection on flag shoots that show deformation. A large number of conidia are produced on these shoots after sporulation and when the wind blows and carried them away to healthy shoots resulting in heavy infection of all healthy tissue of the vine. The second strategy of overwintering *U. necator* was adapted as chasmothecium (i.e. from mid-summer to autumn). Cleistothecium is present on bark, waste berry, and leaves on the soil surface overwinter, and starts sporulation when getting moisture in spring during rainfall. The ascospores in the fungal fruiting body i.e. chasmothecium or cleistothecium released and reach to lower leaves of the vine by wind, or by splashing of raindrops. For the release of ascospore, free water is needed but a constantly moist or wet environment is not mandatory for spore germination subsequently which can cause infections. An appressorium is formed when conidia and ascospores germinate on the infection site. The mechanism of infection involved peg developments that penetrate the lower surface of leaves. These pegs penetrate the skin through the epidermal cell and form haustorium. Within a few days, new conidia are produced when the hyphae nourish on the cuticle, and in this way, *U. necator* sustains its life cycle. Such cycles are repeated many times across the growing season causing rapid augment in the spread of infection [15].

Chemical control

Protection of grapevines from diseases particularly powdery mildew is essential to minimize the economic losses of grapes. To decrease the inoculum potential, a disease management program must be undertaken early in the season which is imperative to reduce late-season disease problems. Because, without early control of the infection of powdery mildew, often lead to severe problem in the late season [16, 9]. The severity of powdery mildew can be minimized by adapting cultural practices like pruning and thinning that facilitate better air drainage and give shape to the canopy in a way to enhance sunlight penetration. However, such cultural practices can't decrease the incidence of disease to the extent that becomes acceptable for the commercial

production of grapevine. Nevertheless, other cultural practices like the removal of leaves integrated with the application of fungicides may have a significant influence on the incidence of powdery mildew [17]. They conducted an experiment to remove leaves and then sprayed fungicides on the grapevine. Their findings revealed that leaf removal along with fungicide sprays resulted in the creation of a microclimate on berries' surfaces that was not suitable for the proliferation of fungal infection. They further observed that the practice of leaf removal might have improved the efficiency of fungicides in terms of their better penetration into the canopy.

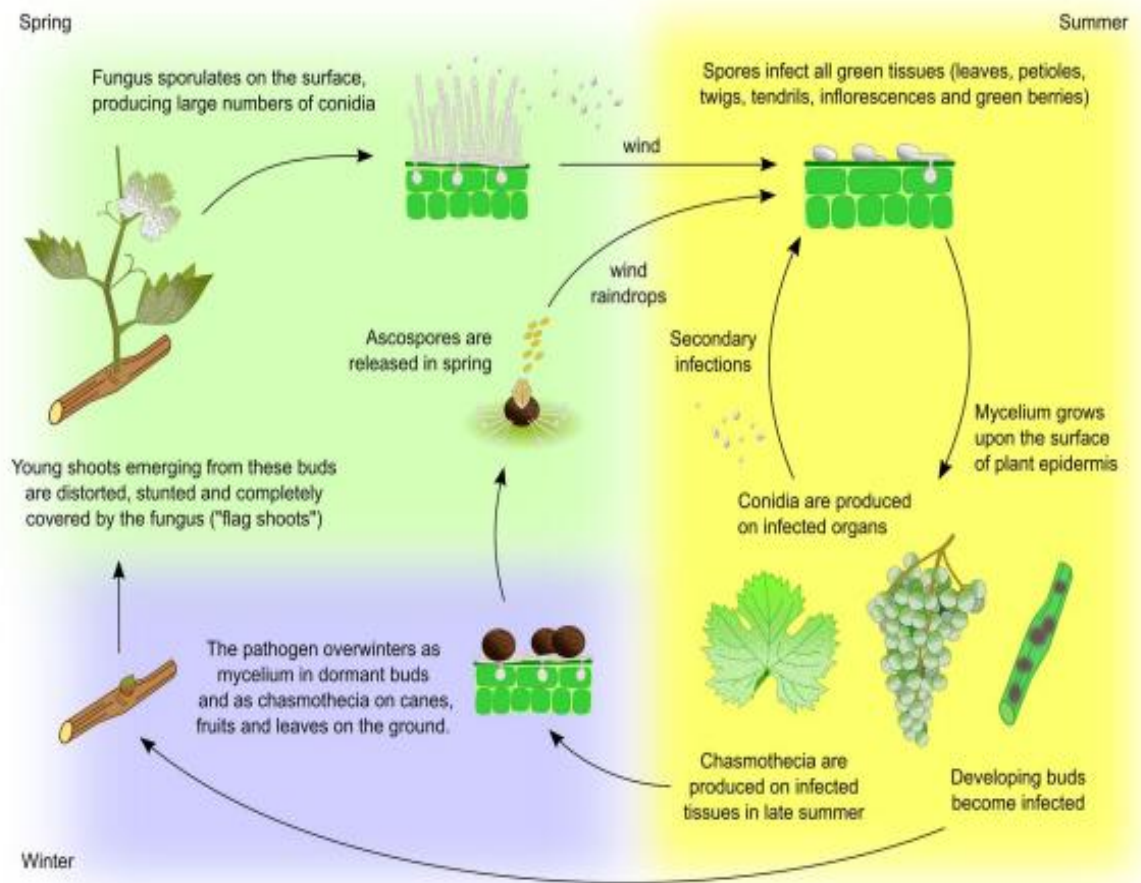


Figure 1. Powdery mildew (*Uncinula necator*) life cycle [15]

Historically, elemental sulfur was used as the first fungicide against fungal disease viz. powdery mildew [9] which is still in use as an effective and cheap fungicide for vineyards. The elemental sulfur thwarts spore germination and is performed as a protective fungicide [18]. For organic vineyard production in California, the use of sulfur is approved for the control of powdery mildew [19].

Sterol biosynthesis inhibitors, also called SI fungicides, are the latest products to control powdery mildew effectively. The mechanism of SI fungicides is involved interference in cell membranes' formation [18]. However, its continuous application has caused resistance to powdery mildew that resulted in its low efficacy. The grapes infected by various strains of powdery mildew when subjected to SI fungicide sprays showed ineffective control that might be due to resistance of some strains to SI fungicides which were observed across grown regions of grapes globally [20]. The resistance against SI fungicides develops quickly in a warm climate when disease pressure is high because high temperature is conducive to fungal multiplication, particularly the resistant strains [21]. It was observed by a group of researchers that fast ascospores formation led to the

production of resistant strains in high numbers [9]. The same results were also testified by [22] that widespread use of SI fungicides has caused resistance to eradicating powdery mildew of grapes. According to many researchers that natural populations of fungi were extremely influenced by the selective utilization of SI fungicide. So, the scheduling of fungicide sprays program set in a way that other than SI fungicides may be included alternatively having a different mechanism of action which is more active against powdery mildew as well as reduces the resistance of strains against SI fungicides [23]. Different strategies can be adopted to sidestep the resistance development in fungal strains of powdery mildew such as reducing the number of sole types fungicide sprays, specifying spray timings, particularly in early season when disease pressure is low and in a single row plant several sprays of sole type fungicides must be avoided that led to control of powdery mildew efficiently [18]. It was further observed by another group of researchers that continuous use of SI fungicides brings changes in plant metabolism in terms of aroma change in fruits at the maturity stage [24].

A recently developed fungicide is strobilurins which are recommended for vineyards on large scale as a broad-spectrum fungicide for controlling many fungal diseases along with powdery mildew. Strobilurins are different from SI fungicides based on their mode of action. Its mechanism of action is to impede spore germination through disruption of the electron transport system in the mitochondria of pathogen that led to unable cells functions to generate energy. Actually, strobilurins are organic compounds having chemical structures that resemble those natural compounds found in wood-rotting fungi. The prominent function of strobilurins is systemic mildly and works for curative activity. Though, being a site-specific mechanism of action, it is extensively used as a preventive fungicide and helps against powdery mildew effectively, and did not develop resistance to the pathogen [18].

A field study was conducted by a scientist to evaluate systemic fungicides and 0.025M K_2HPO_4 and $KH_2PO_4 + KOH$ (both plus Triton X-100) on grapevines, mango, and citrus against powdery mildew. The inhibitory influence of these fungicides was observed on clusters of flowers, fruit, and leaves infected by powdery mildew. Their findings revealed that the foliar sprays of phosphates on berries of Chardonnay grapevines effectively controlled powdery mildew which was statistically at par with the foliar sprays of systemic fungicide of pyrifenoX (Dorado 480 EC). Nevertheless, in comparison to phosphate, the foliar application of systemic fungicides such as diniconazole (Marit 12.5% WP), myclobutanil (Sisthane 12E), and penconazole (Ophir) on mango and nectarine effectively controlled powdery mildew on inflorescence. The use of phosphate along with each of the said fungicides in an alternative way proved high efficacy against powdery mildew in the tested crops. It was further noted that the foliar application of phosphate solutions was not phytotoxic to nectarine leaves except for the newly developed leaves. Consequently, it was inferred that the foliar application of phosphate solutions was compatible and effective against disease control and was also better foliar fertilizer when used on various crops in the field [25].

Different trials were carried out by different researchers during 2002-2004. Three treatments comprising sodium, ammonium, and potassium bi-carbonate were tested in this study. The results indicated an inhibitory influence on disease equally in plants. However, the application of sodium and ammonium in excess quantity manifested problems in grapevines while on the other hand, the use of potassium bi-carbonate was useful which was further for safe utilization. The application of potassium bi-carbonate up to and/or above 100 mgL^{-1} affected conidial germination under in vitro trials. Among different rates of potassium bicarbonate, 10 g L^{-1} was observed effective as a preventive fungicide when sprayed after 40 days of pruning at an interval of 10 days. The same rate of potassium bi-carbonate was found at par with a spray of sulfur when used at the rate of 2.0 g L^{-1} as wettable sulfur powder. Sporulation of the pathogen was reduced by 80% under the influence of a potassium bi-carbonate spray of 10 g L^{-1} when sprayed on the infected leaves. In addition to that, the addition of non-ionic surfactant

augmented the efficacy of potassium bi-carbonate against powdery mildew which might be due to its better coverage on the foliage. The integration of potassium bi-carbonate @ 5 g L⁻¹ with hexaconazole @ 0.5 ml L⁻¹ revealed effective control of powdery mildew over the sole application of hexaconazole. The compatibility of potassium bi-carbonate was tested with fungicides including Myclobutanil, Flusilazole and Penconazole and found satisfactory results demonstrated that integrated use of potassium bi-carbonate with fungicides is compatible and can be used for better and more effective control of diseases particularly powdery mildew [26].

A group of researchers managed the use of three fungicides to control *Erysiphe necator* (Schwein) Burrill induced epidemic in grapevines where the sprays of these fungicides were the continuous practice by grapevine growers. They conducted leaf disc bioassays and in vivo studies to evaluate the three fungicides against *E. necator* and found quinoxyfen (protectant fungicides) less effective in terms of minor inhibition in the development of *E. necator*. However, the fungicides having both protective and curative characteristics such as tebuconazole and trifloxystrobin, produced a significant inhibitory influence on the development of the *E. necator* epidemic. The results of leaf disc bioassays revealed that the fungicides were highly effective at different growth stages of fungi which were noted in terms of the use of tebuconazole was effective before sporulation while the use of trifloxystrobin showed an inhibitory effect after sporulation of fungal colonies. The field studies also indicated similar results as obtained in bioassay studies where the fungal development at various stages was come upon instantaneously. The fungicide applications studied under field conditions pertaining to the occurrence and sternness of disease on bunches showed the effectiveness of fungicides in the inhibition of *E. necator* infection cycles. They suggested the integration of different fungicides as a protective strategy in this study [27].

Kast and Bleyer [28] stated that in grapevine enterprises, the infection caused by the pathogen *E. necator* was the furthestmost disparaging. They ordinarily carried out >7 sprays of different fungicides to the disease controlled across the growing season i.e., up to the stage of veraison. In Germany, the comparison of two fungicide regimes was studied in grapevines between 1999 and 2010. The regimes were comprised of either 3 or 7 fungicide sprays per season. The regime of 3 sprays of fungicides consisted of before flowering, during blossoming, and the third spray at the berries stages when the size of the berry was 2mm in diameter which is also known as the open window period (OWP). While the 7 regimes sprays of fungicides were comprised of OWP sprays along with 1 spray before and 3 sprays afterward. Even though a natural inoculum of the powdery mildew existed during 2008-10, the untreated spacer lines were further supplemented with an artificial inoculum of the pathogen. From this study, they noted that 3 sprays carried out in OWP controlled infection by 90% when the disease pressure was high, which was statistically at par with the conduction of 7 sprays regimes in OWP as a preventive influence on the occurrence of infections [28].

Cacaj *et al.* [29] conducted a two-year experiment during 2010-11 on two grapevines cultivars i.e. Frankovka and Game to evaluate the fungicides' efficacy and methods of application against powdery mildew caused by *Plasmopara viticola* under field conditions at Rahovec, Kosovo. Seven fungicides were tested for their efficacy against *P. viticola* including Quadris, Antracol EP-70, Dithan M-45, Ridomil, Curzate, Bordeaux mixture, copper sulphate) and Mikal respectively. McKinney index was used for monitoring the severity of disease in this fungicide evaluation study. The severity of grape downy mildew was observed in fungicides treatments which were statistically highly significant for both cultivars and years as treatments. Among the evaluated seven fungicide treatments, the integrated use of Ridomil and Dithan M-45 manifested high efficacy against grape downy mildew [29].

A scientist conducted a three-year study (2014-16) to evaluate the effectiveness of fungicides against powdery mildew in the affected vineyard. The quinoxyfen performed equally as that of myclobutanil and boscalid which were statistically at par with each other in the control of

powdery mildew. There is little information available describing the mechanism of action of quinoxifen and it has been noted in previous research studies that quinoxifen disrupts the process of signal transduction. The process of making RNA from DNA through RNA polymerase was studied under the influence of quinoxifen manifested MARK signaling regulation and activities including sporulation and pathogenesis. While another important pathogen of grapes is downy mildew caused by *Plasmopara viticola*, which is effectively controlled when phosphite was sprayed [30].

Ram Reddy *et al.* [31] conducted a field experiment for two years on grape variety Thomson seedless during 2008-09 and 2009-10 evaluated 8 fungicides and integration of fungicides with potassium bi-carbonate along with control against powdery mildew in grapes and also analyzed the terminal fungicidal residues in grape production. The results revealed that spraying of Fusilazole 40EC @0.125ml/l at 40 days after forward pruning followed by– Penconazole10EC @ 0.5ml/l + Potassium bicarbonate @ g/l at 60 days AFPR – Triademefon 25WP @ 1g/l at 70 days AFPR - Hexaconazole 5EC @ 1ml/l + Potassium bicarbonate @ 5g/l at 80 days AFPR – Myclobutanil 10WP @0.4g/l at 90 days AFPR – Azoxystrobin 23SC @ 0.5ml/l at 105 & 120 days AFPR (or) Fusilazole40 EC @0.125 ml/l at 40 days after forward pruning followed by Penconazole 10 EC @ 0.5 ml/l + Potassium bicarbonate @ 5 g/l at 60 days after forward pruning followed by Triademefon 25WP @1.0 g/l at 70 days after forward pruning followed by Hexaconazole 5 EC @ 1.0 ml/l + Potassium bicarbonate @ 5 g/l at 80 days after forward pruning followed by Myclobutanil 10WP @ 0.4 g/L at 90 days after forward pruning + Pyraclostrobin 20%WG @0.5g/l at 105 & 120 days AFPR were found statistically at par in decreasing the PDI on leaf and berries while augmented its saleable production over other six spray schedules and control. Studies on terminal fungicidal residues in grape produce revealed that all treatments were below MRL or below damage level (BDL) [31].

The result of the study evaluated the frequencies of metalaxyl + mancozeb in five treatments based on a randomized complete block design with 3 replications using grapevine cultivar BRS Vitoria under two separate experiments (i. production phase, ii. branches formation phase) at Jales, São Paulo. The frequency treatments were comprised of five treatments such as the application of metalaxyl + mancozeb at intervals of i. two weeks, ii. One week, iii. every 14 days, iv. Every 21 days and v. after sporulation. Three vine plants per treatment were managed under this study. Disease control level was determined through DPC (disease program curve) which revealed statistically significant variations among the treatments and maximum control of disease was achieved when the frequency of metalaxyl+mancozeb sprays was performed at two weekly intervals followed by one weekly interval. In addition to that 90% control was obtained when the mentioned fungicides mixer was sprayed after sporulation over the two weekly spray intervals. This frequency of the fungicide mixtures sprays resulted in control of the powdery mildew and the leaf area that remain affected by <3% inflicted no harm to the growth and fruit production and quality with better grapevines clusters physicochemical properties [32].

Fungicides mode of action

For efficient use of fungicides with no or less resistance to the pathogen, it is appropriate to spray fungicides having a different mechanism of action that is specific in function and for more efficacy, use a mixture of such fungicides that have no harmful impact on plant growth and environment. The application of such specific fungicides damages fungal infection in many ways including prevention of spore germination, colonization, and reproduction at particular or fungal growth formation. And above all, the use of fungicides suppresses or disturbed the fungal's structure and biochemical processes. Diversified mechanisms of actions of fungicides which are known and unknown are included disruption of the cell membrane, cell division, protein synthesis, respiration, and signaling system of the pathogen [33, 34].

Cell membrane components

There is a similarity in the cell membrane of fungi and eukaryotes in terms of structural organization and functions. The major functions of the cell membrane are included conductivity of selective ions and molecules of organic and inorganic nature, passing on signals, maintenance of cell shape, adhesion, and safeguarding cell from hazardous constituents. One of the important components of the cell membrane is sterol membrane lipid that performs biogenesis called ergosterol (ERG). The word ergosterol is derived from ergot (*Claviceps spp.*), and its main function is to maintain the fungal cell membrane permeability and flexibility [35, 36]. Alternative to cholesterol in the animal cell membranes, the ERG, a special cell membrane component of fungus, assists as a specific set target for fungicides. The ERG-specific fungicides disintegrate the plasma membrane and or prevent the biosynthesis of ERG. Such fungicides are recognized and introduced in the world as sterol inhibitors which are highly effective and used on large scale. Sterol inhibitors serve as a protectant that affects fungal growth at different developmental stages. Since their discovery many decades ago, these ERG-specific fungicides have covered one-fifth of the world market because of their broad-spectrum nature and mobility through the plant body. Triazole is one of the sterol inhibitors that suppress fungal growth by attacking the ergosterol specific site mechanism i.e. membrane dismantling and inhibition of fungal infection. However, fungal spores contain a reserve of ERG due to which sterol inhibitors become ineffective and have no influence on spore germination. On other hand, the fungicides group that interfere in the biosynthesis of lipid in fungal cell membrane and suppress the growth of mycelium are hydrocarbon fungicides or AH fungicides. The next generation fungicides that affect the production pathway of ERG are De Methylation Inhibitors (DMI) that prevent the demethylase production which is essential for biosynthesis of ERG and suppress pathogen finally [34, 37-40]. Dicloran and etridiazole are AH fungicides and the mechanism of action involves the cell membrane's lysis through the deterioration of linoleic acid in the membrane and by hydrolysis of phospholipids [41]. Despite that, some fungicides affect the plasma membrane but other fungicides like acriflavine serve as an inhibitor of intracellular membrane i.e. membrane of mitochondria by disrupting its permeability. Such disruption results in changes in the proton gradient across the membrane and make it imbalanced leading to low production ATP that causes the death of fungal cells [42].

Signal transduction

Fungi respond to environmental stimuli and intracellular signal transmission through the mediation of the Mitogen-Activated Protein (MAP) Kinase pathway. For suppression of mycelial growth and spore germination, site-specific fungicides affect the osmotic signaling system in fungi [43]. In addition to that those fungicides interfering with components of cell membrane also inhibit the signaling system at the interface of cytoplasm and plasma membrane [44]. The fungicides targeting the sites of the osmotic signal transduction pathway which are used on a large scale globally against fungal infections are two groups' viz. phenylpyrroles (PP) and dicarboximides [45]. Fludioxonil is another group of PP fungicides that disrupt fungal growth through inhibition of spore germination by interfering with osmoregulatory signaling in *Botrytis spp.* [46, 47]. Another fungicide group called dicarboximide such as Iprodione, reduces fungal growth, and elongation of mycelium along with inhibition of hexose and chitin formation which is due to the failure of sterol [48, 49]. Together phenylpyrroles (PP) and dicarboximides groups affect signal transduction by disrupting of histidine kinase pathway that resulted in the irregular function of MAP for phosphorylation [50].

Inhibition of Respiration

Inhibition of respiration in fungi is driven by disruption of a mitochondrial electron transport system that takes place in respiratory complex 1 of mitochondria when NADH is reduced to

coenzyme Q called ubiquinone [51]. The oxidation-reduction activity of NADH is suppressed by C1 fungicide (complex 1 inhibitor) which ultimately affects fungal respiration. In some ornamental plants, the fungal or rust infection is controlled by using C1 fungicides like diflufenconazole which show its mode of action through targeting an oxidoreductase [52, 53]. The respiratory complex II system performs the succinate oxidation link with coenzyme Q (ubiquinone) which is interpreted by 4 SDH genes) [54, 55]. Fungal infection is inhibited through a reduction in respiration when CII complex fungicides (i.e. boscalid, carboxin, and flutolanil) are sprayed based on their mode of action driven by disruption of SDH functions in electron transport and tricarboxylic [56, 57]. A tremendous yield enhancement was recorded when CII complex or SDH inhibitors were investigated under field conditions [58].

It has been reported by a group of researchers that one of the site-specific fungicides disrupts mitochondrial respiration by targeting the Complex III enzyme which is driven by the inactivation of cytochrome bc₁ at two sites i.e. Quinone outside (Q_o) and Quinone inside (Q_i) (Gisi *et al.*, 2002) [59]. These site-specific fungicides are also called Q_o and Q_i inhibitors because the energy production potential of the fungus is reduced resulting in disruption of respiration and finally the death of the fungus (Mueller *et al.*, 2008) [60]. The fungicides used nowadays on large scale are strobilurin inhibitors that belong to the QoI family [61].

The majority of fungicides whose mechanism of action is based on respiration disruption involve many pathways such as targeting the enzyme complexes, hindering the process of respiration through the upsetting of energy production in the form of uncoupling ATP synthase and oxidation of phosphorylation. Among them, fluazinam adopts the same mode of action that manifested extrication activities comprising glutathione joining, interruption of ATP synthesis as well as upsetting many metabolic activities and pathways [62, 63].

Synthesis of amino acid

The synthesis of protein plays a significant and vital role in various functions in the cell. In fungi, the different functions of protein involve the formation and strengthening of cell membrane and structure of cell wall, sensing the environmental changes, transduction of signals across membrane and mycelium, and other physiological and biochemical reactions. The designed fungicides target the enzymes responsible for the synthesis of the cell wall structure and functions [64, 65]. Fungicides affect protein synthesis by upsetting methionine gene biogenesis. Such protein inhibitors are called AP fungicides (anilino-pyrimidines inhibitors). Some products possess both antifungal and antibacterial characteristics such as Streptomycin, when used for the control of disease affects the biogenesis of protein, and signal transduction process leading to 70S ribosome interruption where isoleucine was added in polypeptide chains [66]. Similarly, other fungicides such as oxytetracycline interrupt amino-acyl complexes of tRNAs on ribosomes that finally target the bacterial population by upsetting the ectoenzyme activity [67].

Interruption in cell division

In all living entities, the sustainability of life is driven by the indispensable biological process involving mitosis and cell division necessary for their reproduction [68]. The mechanism of various antifungal groups disrupts mitosis as well as cell division in fungi at different phases [69, 70]. The fungicides including methyl benzimidazole carbamates hinder paths of tubulin monomers counter in an integrated manner for creating polymers of microtubule [71]. In addition to that benzimidazoles, consisting of carbendazim, benomyl, and thiabendazole compounds, affect the buildup of β 2 tubulin recombinant up to 93.5% that ultimately interrupt the process of polymerization and death of fungus through interruption of mitosis particularly in *Fusarium spp.* Likewise, benzimidazole's mode of action prevents the formation of microtubules but has low efficacy against spindle microtubules/polymerized cytoskeleton [72]. The main function of

spindle microtubules remains to arrange chromosomes in a direct manner at the metaphase plate. The instability between spindles and kinetochore is due to loss of chromatids when such mitosis inhibitors are applied [73].

Inhibiting synthesis of nucleic acid

The inhibitors targeting the biogenesis of nucleic acid are phenylamines (PA) fungicides group that comprised of several antifungals having target site of RNA polymerase I [74]. Likewise, the incorporation of uridine interrupts the RNA chain through the application of metalaxyl. It was further observed that the synthesis of RNA is severely affected as compared to DNA by the application of PA inhibitors [75]. During the biosynthesis of nucleic acid, the uridine transcription process is affected by the intrusion in RNA transferase systemic activities. Further, the use of ethirimol which is a member of the hydroxyprimidine group, inhibits adenosine deaminase activity. The nucleotide function as adenine and inosine as a metabolic agent is also affected by the ethirimol [76]. It interrupts the balance of the nucleotide pool through increasing adenine regains the path of phospho-ribosyl-transferase and hinders the action of adenosine deaminase that led to terminated biogenesis of inosine as well as reduced production of nucleic acids [77]. The heteroaromatic group of fungicides such as hymexazol disrupts the biosynthesis of DNA or RNA [78]

Multisite activity

The most extensively used fungicides in agriculture are multisite inhibitors, nonetheless, inflict detrimental *consequences* on non-target populations. A fungicide like Chlorothalonil is cogitated as an effective inhibitor because of multisite biochemical targets that decrease the enzymatic action and prevent the conversion of glutathione into different chemical structures [79]. Mancozeb is another fungicide having different sites of action that disrupt the growth of fungus which is widely used in agriculture [80]. Many fungicides such as thiram, captan, propineb, maneb and other copper-based products are applied due to their multisite actions [81].

Broad-spectrum fungicides

The inhibition efficiency of fungicides concurrently against various pathogens is called contact/systemic fungicides. The use of such fungicides also acts for diverse hosts then such inhibitors are termed broad-spectrum fungicides. In a situation when the disease-causing pathogen is unknown or a complex of disease agents exists, then the use of a broad-spectrum fungicide is highly efficacious. The first broad-spectrum fungicides registered in 1962 were mancozeb and dithio-carbamate and since the late 1970s, used in 120 countries across the globe they were the primary active ingredient with greater sales in 2004. After tebuconazole, mancozeb recorded the second maximum sale of 500 million US dollars in 2007. The expansion, commercialization, and use of mancozeb on various crops with infected diseases resulted in different formulations. Its spectrum can further be broadened if co-formulation is integrated, then in 2007 fetch 740 million US dollars from its business [82]. According to a group of researchers that because of reproductive and developmental toxicity, the use of mancozeb in Europe has again been brought under review to better understand its impact on hosts and other non-target groups [83]. Azoxystrobin is broad spectrum fungicide that played a significant role in agriculture and is considered a model fungicide. The importance of these fungicides prevailed through their registration on more than 80 crops across different nation state and has shown efficacy against fungal diseases. It has been reported by many researchers that the majority of fungicides that are members of the strobilurin group have broad-spectrum properties and their inhibition capacity varies in respect of host, rate, and time of applications. Some well-known strobilurin members are kresoxim methyl, metominostrobin, and trifloxystrobin. The diseases caused by fungi belonging to the class oomycetes are moderately

or poorly controlled by trifloxystrobin and kresoxim-methyl but effective control was observed when metominostrobin was used against the pathogens of oomycetes that have caused diseases in rice and turf hosts [84, 85]. The fungal diseases of apples, grapes, and strawberries were effectively controlled by using broad spectrum fungicide “Benzovindiflupyr” and its efficiency was augmented when integrated with other fungicides [85,86].

Conclusion

For better control of powdery mildew (*Uncinula necator*) in grapes, it is imperative to apply the right fungicides at the right time. From this review, it was inferred that both protective and curative fungicides were proved highly effective against *U. necator* when sprayed one week before flowering which resulted in greater grape yield with healthy berries. After the bloom stage, the use of systemic fungicide revealed better results when sprayed at the stage of berry formation. So, for effective control of *U. necator*, a protective spray of fungicide before bloom and a subsequent spray of systemic fungicides at the time of berry formation ensure healthy and higher grapes yield. The selection of fungicides depends on their mode of action as well as their nature of formulations in an integrated manner keeping in view the creation of resistance to fungal pathogens. The use of broad-spectrum fungicides is effective against many pathogens infecting different host crops.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No animals were used in this study. The study on humans was conducted in accordance with the ethical rules of the Helsinki Declaration and Good Clinical Practice.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

None.

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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