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REVIEWS

Overview of methods and considerations for the photodynamic inactivation of microorganisms for agricultural applications

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Abstract
Antimicrobial resistance in agriculture is a global concern and carries huge financial consequences. Despite that, practical solutions for growers that are sustainable, low cost and environmentally friendly have been sparse. This has created opportunities for the agrochemical industry to develop pesticides with novel modes of action. Recently the use of photodynamic inactivation (PDI), classically used in cancer treatments, has been explored in agriculture as an alternative to traditional chemistries, mainly as a promising new approach for the eradication of pesticide resistant strains. However, applications in the field pose unique challenges and call for new methods of evaluation to adequately address issues specific to PDI applications in plants and challenges faced in the field. The aim of this review is to summarize in vitro, ex vivo, and in vivo*/in planta* experimental strategies and methods used to test and evaluate photodynamic agents as photo-responsive pesticides for applications in agriculture. The review highlights some of the strategies that have been explored to overcome challenges in the field.

Keywords Antimicrobial photodynamic inactivation · Photosensitizer · In vitro · In vivo · *In planta*

1 PDI principle

Photodynamic inactivation, also referred to as antimicrobial photodynamic inactivation (aPDI), uses light-responsive molecules (photosensitizers, PS) that when excited by light react with molecular oxygen to generate reactive oxygen species (ROS) [1]. ROS can be highly cytotoxic if produced in sufficient amounts and in close proximity to the pathogens as they can cause irreversible damages to both the outer membranes as well as internal structural and cellular components (proteins, lipids, DNA, membranes, cytoskeleton), culminating in microbial death [2, 3]. Depending on the type of PS, generation of ROS can follow two different photochemical mechanisms. Upon absorption of a photon by the ground-state (inactive) PS, the singlet excited state $^1PS^*$ is formed. Intersystem crossing converts a portion of this state into the triplet state ($^3PS^*$) which can then interact with molecular oxygen by either electron transfer (Type I photosensitisation), or energy transfer (Type II photosensitization) (Fig. 1). In the former, the interaction between the activated electron and a molecular oxygen leads to the formation of ROS, such as hydrogen peroxide (H_2O_2), superoxide ($O_2-\bullet$) or hydroxyl radical (•HO); in the latter, singlet oxygen ($^1O_2$) is produced [4–6] (Fig. 1). While both Type I and II photosensitization can occur simultaneously upon excitation of a PS, in general the Type II reaction is preferred in aPDI [7, 8]. The high reactivity of $^1O_2$ is partly due to its lifetime (3–50 ms in aqueous media and several tens of ms (2–1000 ms) in lipid environments such as membranes), which allows this species to diffuse over relatively long distances before being deactivated [9, 10]. If

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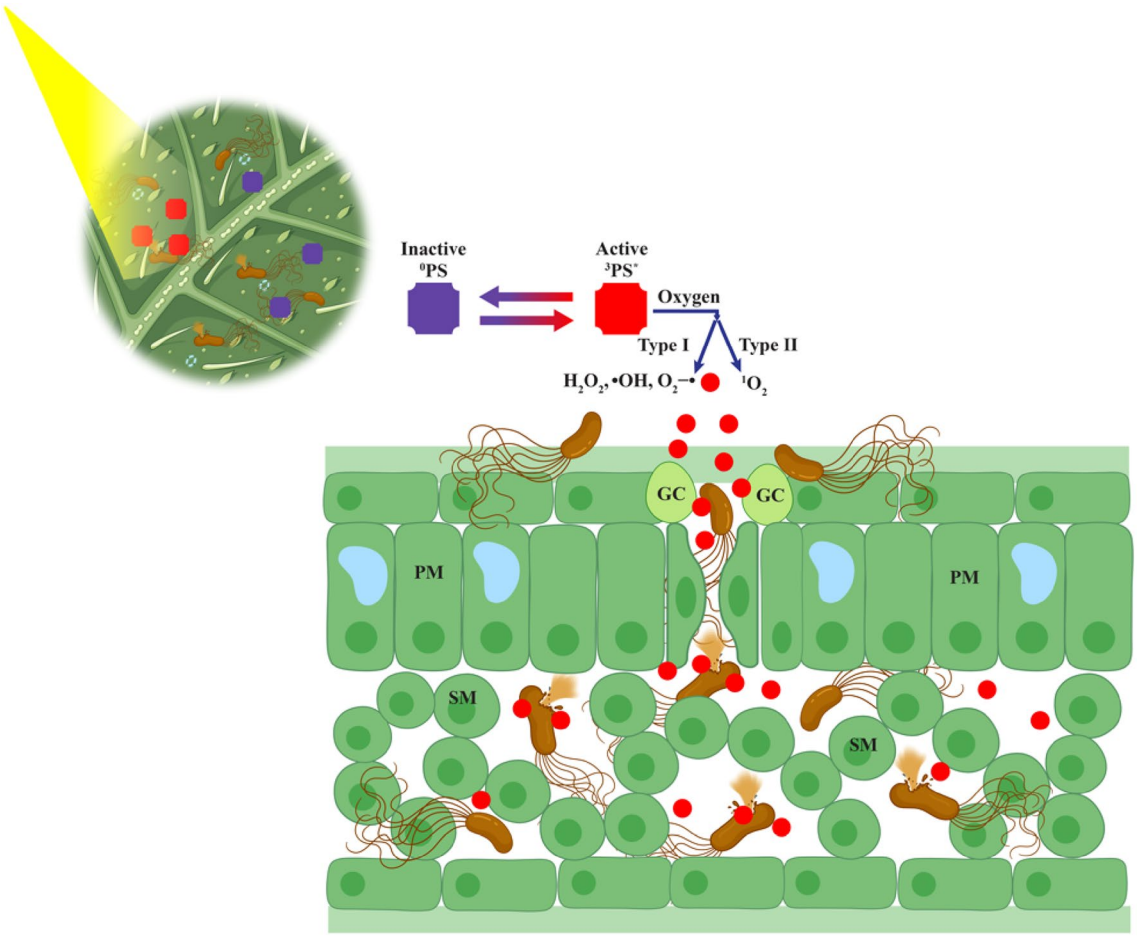

Fig. 1 Schematic representation of aPDI on a plant leaf; viewed in cross-section. PS molecules entering through stomatal openings. *GC* guard cells, *PM* palisade mesophyll, *SM* spongy mesophyll

generated in the apoplast, singlet oxygen can diffuse across the cell membrane [11], where it can last long enough to interact with its targets. While ROS can be non-specific in their interactions with pathogens and host membranes, toxicity towards plant cells has been observed only at high concentrations whereas microbial inactivation is effective at micromolar concentrations [12–14]. Several studies have shown the non-toxic properties of PSs against various plant species at photochemically active doses [15–17] as well as no toxicity upon repeated applications in developmentally-relevant plant stages [18]. Having said that, sensitivities to a PS can be species-specific and similar concentrations may have contrasting-impacts. This might be linked to structural differences in the cuticle/wax layer, or antioxidative capacity in cells. For instance, cationic porphyrins, were non-damaging to tomato plants whereas they completely eradicated the model plant *Arabidopsis* [19, 20].

The wavelength of light required for activation depends on the absorption characteristics of the PS used, but for agricultural uses PSs that can absorb a substantial portion of photons in the photosynthetically active radiation of the solar irradiance. Generally, antimicrobial activity of PSs seems to be more efficient if natural sunlight is used, likely because of its higher intensity as well as its use of the full absorbance spectra of the molecules [21–23]; however, antimicrobial activity is also effective in greenhouse settings with commercially available LEDs [15, 21–23]. Several types of PSs have been shown to be effective against plant pathogenic bacteria and fungi, as summarized in Table 1. Nonetheless, the efficacy of such PSs is typically assessed in traditional liquid culture, which differs significantly from killing pathogens on the surface or within the body of a plant leaf. In the following section, three key challenges associated with such predictions are outlined as well as descriptions of protocols that aim to address these challenges.

Table 1 Summary of photosensitizers used against plant pathogens

Species	*Method of application*	Photosensitizer used	Light condition, exposure time	References
Fungi				
Alternaria alternata	In vitro and in vivo (samples of tomato, squash, pepper and cucumber were collected from the field)	Toluidine blue O (TBO)	White light lamp, 400W/m^2, 60 min	[43]
*Alternaria solani*and *Botrytis cinerea*	In vitro and in vivo (strawberry plants)	Sodium magnesium chlorophyllin	395 nm, 26.6 J cm^{-2}, 120 min	[18]
Aspergillus flavus	In vitro and in vivo (maize kernels)	Curcumin	In vitro: 420 nm, 0, 12, 24, 60 and 84 J cm^2 In vivo*:* 420 nm, 60 J cm^2, unspecified exposure time	[44]
Aspergillus flavus conidia	In vitro and in vivo (maize kernels)	Curcumin	420 nm, 60 J/cm^2, unspecified exposure time	[45]
Aspergillus flavus spores	In vitro and in vivo (maize kernels)	Curcumin	430 ± 3 nm, 10.4, 28.9 and 36.2 mW/cm^2 irradiances tested, 104.2 J/cm^2 optimal light dose (0–130.3 J/cm^2 tested), 30 min	[46]
Aspergillus flavus, Trichotheciumroseum, Fusarium avenaceum, Rhizopus oryzae	In vitro	Hematoporphyrin dimethyl ether	30 mW/cm^3, 15 min	[47]
Bacillus subtilis and *Fusarium oxysporum*	In vitro	Chlorin e6	5 W, 5700 k, 4000 lx; OPPLE Lighting, Shanghai, China	[48]
Botrytis cinerea	In vitro	ZnO nanoparticles	96 W/m^2, 30 min	[49]
Botrytis cinerea	In vitro	Curcumin	432 nm, 120 J/cm^2 (500 W xenon arc lamp), 10 min optimal conditions	[50]
Botrytis cinerea	Ex vivo detached grapevine leaves	Porphyrin	White lamp, photon flux density of 120 µmol/m^2s^1, 16 h	[51]
Botrytis cinerea	In vitro and in vivo (wheat sprouts)	Photoactivated chlorophyllin-chitosan complex (Chl-KCHS)	In vivo*:* 405 nm, 38 J/cm^2, 30 min In vitro*:* 405 nm, 9.6 mW/cm^2 (LED)	[52]
Cladosporium cucumerinum	In vivo	Bengal rose, toluidine blue, and methylene blue	Eight Na-luminescent lamps (unspecified wavelength), 150–190 µmol m^{-2} s^{-1}, 12 h	[53]
Colletotrichum abscissum	In vivo and ex vivo on petals and leaves of sweet orange (*Citrus sinensis*) in different seasons and weather conditions	Methylene blue	solar radiation [(45.8, 579.2, spring), (37.1, 482.2, summer), (20.1, 299.7, autumn), (40.0, 401.7, winter); Format: (UV irradiance (W/m^2), spectral irradiance (W/m^2), season)], 30 min	[54]
Colletotrichum acutatum	In vivo (on *Citrus sinensis* leaves) and in vitro	Furocoumarins and coumarins	Solar radiation, 1 h	[55]
Colletotrichum acutatum and the ascomycete *Aspergillus nidulans*	In vitro and in vivo (*C. sinensis*/orange tree and Murcott tangerine leaves)	Coumarins and furocoumarins	Solar radiation, 1 or 2 h	[56]
Colletotrichum acutatum, Colletotrichum gloeosporioides, and *Aspergillus nidulans*	In vivo (*Citrus sinensis* leaves)	Phenothiazinium derivatives (methylene blue, new methylene blue N, toluidine blue O, and pentacyclic phenothiazinium photosensitizer S137)	634 nm artificial light, 9.2 mW/cm^2, increments of 9 min up to 54 min Solar radiation [(20.7, 3.7, midautumn), (18, 2.9, late autumn) Format: (Spectral irradiance (mW/m^2), UV irradiance (mW/m^2), season)], 1 and 2 h	[57]

Table 1 (continued)

Species	*Method of application*	Photosensitizer used	Light condition, exposure time	References
Colletotrichum graminicola	In vitro	Five cationic meso -(1-methyl-4-pyridinio) porphyrins	Quartz/halogen lamp,0, 30, 60, 90, 120 J/cm^2, 20 min	[58]
Cryptococcus neoformans	In vitro	Polycationic conjugate of polyethyleneimine and the photosensitizer chlorin(e6)	665 nm, 100 mW/cm^2 with fluence from 0 to 16 J/cm^2, 2.67 min	[59]
Cryptococcus neoformans melanized cells	In vitro	ClAlPc in nanoemulsion (ClAlPc/NE) (a phthalocyanine)	Unspecified light source, 5 and 10 J/cm^2, exposure time not specified	[60]
Fusarium and *Penicillium*	In vitro	α-terthienyl (α-T), 8-methoxypsoralen (8-MOP)	UV-A source—40–43 J/m^{2}s^1, UV-B source—1.1 J/m^{2}s^1, UV-C source—1.7 J/m^{2}s^1, no specific exposure time specified but all trials deemed light exposure to be "ineffective"	[61]
Fusarium culmorum	In vivo *(Bidens pilosa)*	Phenylheptatriyne	Solar stimulating Vita light, 200 W/m^2 (5 W/m^2 measured to penetrate sample), 16 h daily	[62]
Fusarium oxysporum	In vitro	Phenylphenalenonephytoalexins (PN3, PN4, PN6, PN8 and PN9) and synthetic phenalenones (PN1, PN2, PN5 and PN7)	18W light bulb (Sylvania, ES Standard, Daylight 154), 15 h	[63]
Fusarium oxysporum, F. moniliforme, and *F. solani*	In vitro	Methylene blue (MB), toluidine blue O (TBO), new methylene blue N coupled with red light (NMBN) and the phenothiazinium derivative S137	635 nm LED, 9.8 mW/cm^2, fluences of 10, 15, or 20 J/cm^2, 30 min	[64]
*Fusarium poae*and *Fusarium culmorum*	In vitro	Protoporphyrin IX	150-W halogen lamp, 150 W/m^2, 30 min	[65]
Gibberellapulicaris (anamorph: *Fusarium sambucinum*)	In vitro	Tested fungi tolerance to furocoumarins	No exposure to light during testing (incubated solely in dark), 7 days	[66]
Harmful molds	In vitro and in vivo (surface of strawberries)	Chlorophyllin-chitosan complex	Visible light ($\lambda = 405$ nm), 19 J/cm^2 for 30 min or 38 J/cm^2 for 60 min	[67]
Lasiodiplodiatheobromae	In vitro	Methylene blue, MB; toluidine blue O, TBO), riboflavin and a cationic porphyrin (Tetra-Py+-Me)	Artificial light, 25 W/m^2, 7 days	[68]
Lasiodiplodiatheobromae	In vitro	Methylene blue, MB; toluidine blue O, TBO), riboflavin and a cationic porphyrin (Tetra-Py+-Me)	Sunlight and artificial Photosynthetic Active Radiation (PAR) (380–700 nm), 25 W m^{-2}	[68]
Lasiodiplodiatheobromae	In vitro	Phenothiazine dyes (methylene blue, MB; toluidine blue O, TBO), riboflavin and a cationic porphyrin (Tetra-Py+-Me)	Natural sunlight, PAR 600 W m^{-2} or an array of 13 380–700 nm fluorescence lamps, 25 W m^{-2}, unspecified exposure time	[68]

Table 1 (continued)

Species	*Method of application*	Photosensitizer used	Light condition, exposure time	References
*Metarhiziumanisopliae*and *Aspergillus nidulans*	In vitro	Methylene blue (MB) and toluidine blue (TBO)	Visible light exposure: 300 W halogen lamp, 50 W/m^2; Laser light exposure: 625 nm, 20 J/cm^2 at 78 W/m^2, 30 or 60 min	[69]
Penicillium chrysogenum	In vitro	Cationic porphyrins	White light, 200 mW/cm^2, 20 min	[70]
Penicillium chrysogenum conidia	In vitro	Cationic porphyrin groups synthesized from 5,10,15,20-tetrakis(4-pyridyl)porphyrin and 5,10,15,20-tetrakis(pentafluorophenyl)porphyrin	White light, 200 mW/cm^2, 20 min	[70]
Phytophthora citrophthora	In vivo pear seedlings and in vitro	Fagopyrin and hypericin	Light conditions not specified	[71]
Sclerotinia sclerotiorum, Pythium aphanidermatum, and *Botrytis cinerea*	In vitro (tested with the bacterial species) and in vivo (cucumber plants tested with the fungal species)	Porphyrin metal–organic framework (MOF) nanocomposite incorporating 5,10,15,20-tetrakis(1-methyl-4-pyridinio) porphyrin tetra(p-toluenesulfonate) (TMPyP)	Natural light or a LED light, 12 W, irradiance 18 mW cm^{-2}, 3–4 days	[72]
Unspecified fungal growth	In vivo (strawberries)	Curcumin	420 nm, 42 J/cm^2, 10 min	[73]
Bacteria				
Erwinia amylovora resistant to *Streptomycin*	In vitro	Sodium magnesium chlorophyllin, chlorin e6 derivative B17-0024	395 nm, radiant exposure 26.6 J/cm^2, 15:50 min	[74]
Pseudomonas syringae DC3000, *Xanthomonas spp,*	In vitro and in vivo	Sodium magnesium chlorophyllin	PAR light at 275, 550 and 1,000 µmol m^{-2} s^{-1}, exposure time 1 h, 16 h, and 5–7 days	[15]
Pseudomonas syringae pv. *actinidiae*	Ex vivo (kiwifruit leaves), and in vitro	5 cationic porphyrins based on tri-cationic porphyrin	In vitro*:* artificial PAR light, 4.0 mW/cm^2, 10, 15, 20, 30, or 60 min Ex vivo*:* artificial PAR light, 4.0 mW/cm^2 for 30, 60, or 90 min *or* 23 mW/cm^2 (sunlight) for 90 min *or* 60 mW/cm^2 (sunlight), 90 min	[17]
Pseudomonas syringae pv. *actinidiae*	Ex vivo (kiwifruit leaves) and in vitro	Porphyrin Tetra-Py + -Me	In vitro*:* artificial PAR light, 4.0 mW/cm^2, 0, 30, 60, 90, 120, 150, 180, or 270 min Ex vivo*:* artificial PAR light, 4.0 mW/cm^2 *or*, LumiCare L122 illumination system, 150 mW/cm^2, or sunlight, 65 mW/cm^2, 0, 30, 60 or 90 min	[37]
Pseudomonas syringae pv. *actinidiae*	In vitro and in vivo (kiwifruit pollen assays)	New Methylene Blue (NMB) and Methylene Blue (MB) with or without potassium iodide (KI)	White light from an aLED projector, 50 mW cm^{-2}, 90 min	[75]
*Pseudomonas syringae*pv. *lachrymans* and *Clavibactermichiganense* subsp. *michiganense*)	In vitro (tested with the bacterial species) and in vivo (cucumber plants tested with the fungal species)	Porphyrin metal–organic framework (MOF) nanocomposite incorporating 5,10,15,20-tetrakis(1-methyl-4-pyridinio) porphyrin tetra(p-toluenesulfonate) (TMPyP)	Natural light or a LED light, 12 W, irradiance 18 mW cm^{-2}, 3–4 days	[72]

Table 1 (continued)

Species	*Method of application*	Photosensitizer used	Light condition, exposure time	References
Rhodococcusfascians, Gram *Xanthomonas axonopodis* and *Erwinia amylovora*	In vitro and ex vivo	Anionic sodium magnesium chlorophyllin with cell wall permeabilizing agents (Na_2 EDTA or polyaspartic acid sodium salt (PA)) and B17-0024, a mixture of chlorin e6 derivatives with cationic moieties	395 nm, 14.8 mW/cm^2, 26.6 J/cm^2, 30 min	[31]
Xanthomonas citri, subsp. *citri*(Xcc)	In vivo disease incidence, puncture leaf and infect citrus Honey murcott plants, photostability in wet and dry, ROS, ascorbic acid	2,6-diiodo-1,3,5,7-tetramethyl-8-(P-benzoic acid)-4,4′-difluoroboradiazaindacene (DIBDP)	520 nm, LM-LED, 43 mW/cm^2 or 80 mW/cm2 (solar simulator), up to 10 min	[16]
Xanthomonas citri, subsp. *citri*(Xcc)	Ex vivo (citrus leaves) and in vitro	Toluidine blue O (TBO)	Artificial light, 150 mW/cm^2, 60 min Natural sunlight, 23–60 mW/cm^2, 240 min	[76]
Xanthomonas gardneri	In vivo (tomato seeds)	Methylene blue, toluidine blue, and a combination of both dyes	652 nm, 12.21 mW/cm^2, 20 min	[77]

2 Photostability

While activation of PSs with high intensity sunlight leads to effective antibacterial activity, it can cause rapid photodegradation/bleaching of PS molecules resulting in loss of their absorption and emission. Destruction of the molecules on one hand reduces their effective lifetime [3, 24–26]; on the other hand, for example for chlorophyll derived photosensitizers with degradation products that can be metabolized by the plant [27], it has a chance to prevent the accumulation of compounds in the environment. This reduces or eliminates pesticide residue issues that plague conventional pesticides and limit when such pesticides can be used in the growing cycle. Classically, photodegradation studies are done in liquid through monitoring changes in the absorption spectrum of the photosensitizer (i.e., the appearance of new absorption bands or decrease of the maximum absorption peak) [28]. In agricultural applications however, foliar sprays typically dry on the leaf surface in minutes and remain dry until dew or rain rewets them. This may lead to changes in the aggregation state of photosensitizing molecules. Recently, Islam et al. studied the stability and efficacy of the water-soluble semisynthetic derivative of chlorophyllin (sodium magnesium chlorophyllin (Mg-chl) following dry-rewet cycles mimicking conditions similar to the field [15] (Fig. 2). They observed that, there was a gradual decrease of fluorescence and single oxygen production with time, indicating gradual degradation of the compound under light exposure. However, despite some degradation, the compound was still able to eradicate bacteria significantly (3log10 reduction) up to 5 days after of light exposure, suggesting that the activity of the PS was maintained for at least up to 5 days in the dry state.

3 Contact/bacterial proximity

Given that photo-pesticides are contact pesticides, the proximity of PS to the pathogen is one of the crucial factors determining antimicrobial efficacy since the probability of ROS reacting with target structures and molecules drastically decreases with distance [29]. For example Gram (−) bacteria are wrapped in a densely packed protective layer (outer membrane) comprising mainly glycolipid lipopolysaccharides and phosphoglycerides that serve as a permeability barrier which can effectively inhibit penetration of pesticides into the cell [30]. While both anionic and cationic photosensitizers alone can kill efficiently Gram (+) bacteria in vitro [31], for complete eradication of Gram (−) bacteria a synergistic combination of PS combined with a membrane disrupting molecule is necessary [15, 31–33]. Uptake of anionic PS by bacterial cells may be mediated through

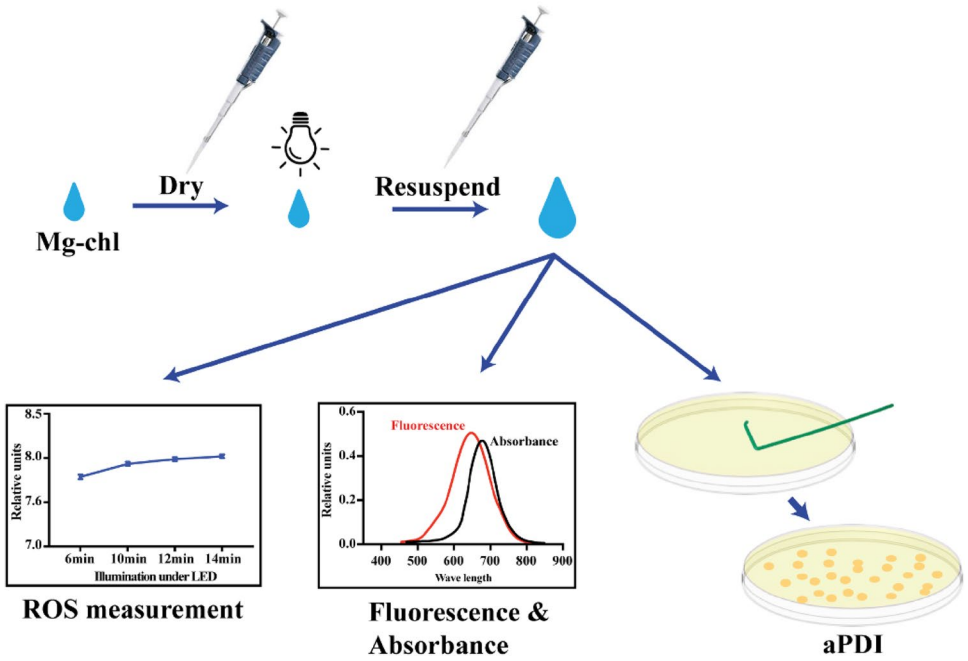

Fig. 2 Schematic diagram of the dry drop method. A droplet of PS is dried in the dark before exposing it to light. After irradiation, the dry PS droplet is resuspended in water and used to measure absorbance, fluorescence, ROS, and antibacterial activity

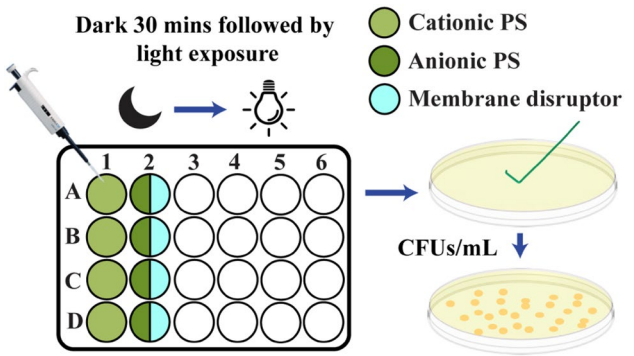

Fig. 3 A schematic diagram of an in vitro aPDI assay. A bacterial culture of a known concentration (OD_{600}) and PS are added in the wells and kept in dark for 30 min to allow the PS to bind/penetrate bacteria, followed by light exposure for 1 h. A membrane disruptor is added with anionic PS in case of treating Gram (−) bacteria. Finally, appropriate dilutions of the suspension are spread on LB agar media to count colony forming units (CFUs)

both protein transporters and electrostatic charge interaction, while the uptake of cationic PS is presumed to be facilitated through self-promoted uptake in addition to interactions with membranes [28, 34]. Efficacy in vitro is typically tested in liquid culture (Fig. 3), with plates kept in the dark after PS addition to allow the PS to adhere to bacterial cell walls prior to light exposure [8, 15, 31]. Although the assay is adequate for determining effective concentration ranges of PSs against pathogenic bacteria in vitro, it does not represent the environment on plant surfaces. The epidermal cells of most plant surfaces are covered with cuticle, a lipid-rich layer that prevents water loss and protects plants against multiple biotic and abiotic stresses [35, 36]. To reflect interactions of bacteria with the cuticle and test aPDI on plant surfaces, both ex vivo experiments using detached plant leaves [17, 37] and in vivo/*in planta* experiments using intact plants have been developed [15]. In ex vivo experiments, detached leaves are sprayed with bacteria and PS (Fig. 4a); the in vivo method follows the same procedure except that bacteria and PS are applied through a foliar spray directly on the plant (Fig. 4b) [15], representing conditions in the field.

In the case of fungi, both anionic and cationic PS can efficiently eradicate the pathogens (refer to Table 1 and references therein). Fungal mycelia typically grow in 3-D structures that often limit access of the PS to all mycelia. To simulate the 3-D structure of mycelia, Hamminger et al., carried out in vitro aPDI of fungi using mycelial spheres in 24-well plates (Fig. 5) [18] and showed that the PSs can efficiently kill fungal species.

4 Light intensity

Exposure to a sufficient amount of light for PS activation is an indispensable requirement for PDI. Particularly in foliar pathogens, the first step in pathogenesis is the colonization of aerial tissue surfaces. Flat surfaces such as cucumber, tomato, and lettuce leaves provide excellent light exposure, and good microbial reduction was observed when the cationic curcumin derivative SACUR-3 was used against *E. coli* O157:H7 [38]. Bacterial pathogens can gain access to internal plant tissues either through wounds or through natural openings such as stomatal pores used for gas exchange;

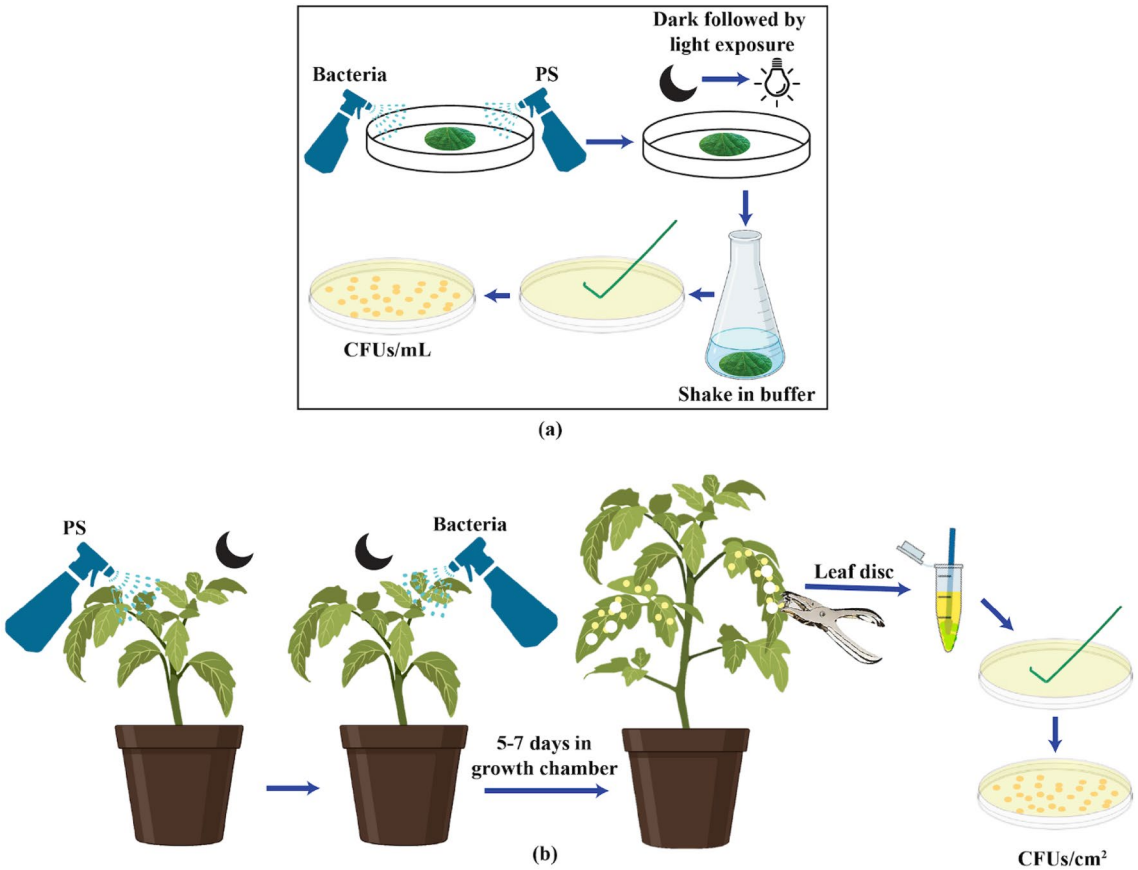

Fig. 4 Schematic diagram of the ex vivo and in vivo aPDI assays. **a** Ex vivo: Bacterial cultures of known concentration (OD_{600}) and PS are sprayed on detached leaves and kept in dark for an hour before exposing them to light for several hours. The leaves are then placed in buffer and shaken to dissociate the bacteria, and an appropriate dilution of bacteria is spread on solid LB media for colony forming units (CFU) count. **b** In vivo*:* PS is sprayed on the leaves of intact plants followed by a bacterial suspension spray and kept in the dark for an hour before plants are transferred to a growth chamber. After 5–7 days, disease severity is assessed, and leaf discs are collected for CFUs count

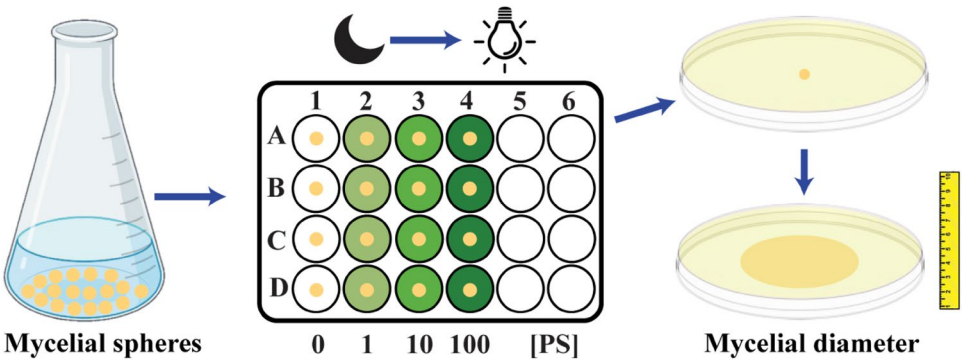

Fig. 5 Schematic diagram of an in vitro PDI assay on fungi. Mycelial spheres and PS are added to 24-well plates and kept in the dark before exposure to light for several hours. Fungal balls are then transferred to media and checked for growth after a few days depending on the growth rate of the specific fungi

alternately, pathogens can be delivered into plants by insect vectors [39, 40]. Until recently it was unclear whether a sufficient amount of light is available to activate PS molecules inside the leaf. Islam et al. investigated this question by infiltrating a mix of bacterial suspension and photosensitizer in the intercellular leaf spaces of tomato and *N. benthamiana* plants, exposing the plants to light, and determining the number of viable bacteria in the leaves after treatment (Fig. 6) [15]. They found a significant inhibition of bacterial growth *in planta* when plants were exposed to light which suggests that PS can be activated inside the leaves and is able to kill intracellular bacteria.

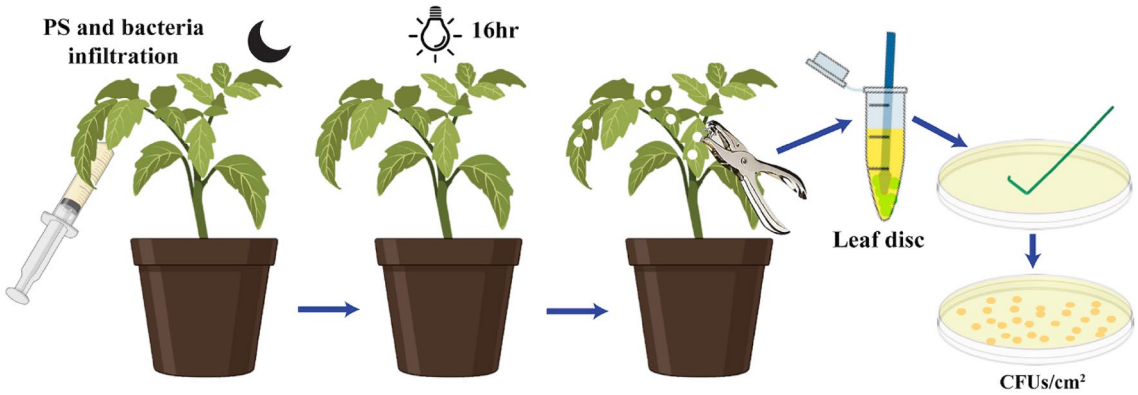

Fig. 6 PS and bacterial suspension are infiltrated in leaves. Plants are kept in dark for an hour before they are exposed to light for 16 h. Leaf discs are collected, ground and bacteria are plated onto media for CFUs count

5 Conclusion and outlook

The photochemical efficiency of aPDI in lab test conditions depends critically on the properties of the PS molecule (e.g. the presence or absence of charge and the charge distribution), the efficiency of light absorption, and the longevity of the triplet excited state or singlet oxygen and free radical production [4]. However, when targeting plant pathogens in vivo, consideration of an additional layer of requirements depending on the lifecycle of the pathogen and tissues it inhabits is important. The design of the photosensitizer must consider the presence of peripheral functionalizations that may confer specific localization in the plant (i.e. hydrophilicity vs. lipophilicity, ability to translocate and/or bind to various cellular structures [41], as aPDI activity may change depending on cell location and/or the oxidative state of the cell. Nanoencapsulation of PSs that can facilitate improved photostability, targeted tissue penetration, and tunable PS release kinetics offer a promising route to address these challenges [42]. In addition, while progress has been made to better represent processes occurring in the field via ex vivo, or *in planta* assays, further development is necessary to address the various challenges often occurring at once in the field-from water fluctuations to intense sun and wind.

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Declarations

Conflict of interest The authors declare no conflict of interest.

Ethical approval Not applicable.

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