

# Perspectives on Anaerobic Soil Disinfestation for Weed Management

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## Abstract

Anaerobic soil disinfestation (ASD) is a pre-plant soil modification method in which soil is amended with easily degradable organic matter followed by saturation with water and covering with plastic sheeting for several weeks to create anaerobic conditions. This process promotes stale seedbed conditions and encourages seed germination. In time, changes in soil physical, chemical, and biological conditions kill the weed seeds and seedlings. Anaerobic soil disinfestation has been used to suppress soilborne plant pathogens. Studies indicate that ASD can contribute to weed management in production systems where pesticides are not permitted or are economically prohibitive. Although most available literature focuses on plant pathogens, in this review we have consolidated the information on the efficacy of ASD for weed management, using examples from widely distributed weed species. We also pose a potential mechanism of action for weed seed mortality due to ASD treatment. Potential areas of research for refinement and validation of ASD for weed management for its broader adaptability have also been described. Finally, we elaborate on the potential of cover crop incorporation in ASD for weed management in specialty crops.

**Key words:** weed, reductive soil disinfestation, bio-solarization, organic production, nonchemical methods

Large-scale conventional farming has long relied on synthetic herbicides for weed management due to high efficiency, ease of use, and low labor requirements. However, the effects of synthetic herbicides on the environment and human health are of considerable public concern (MacLaren et al. 2020). Synthetic herbicides are not allowed in organic production systems, and farmers consider weeds to be the most significant barrier to organic farming (Kristiansen et al. 2001, Birzer and Badgery 2006). The increasing use of synthetic herbicides in weed management has further increased concerns about the emergence of resistant weed biotypes, water pollution, and weed species shifts (MacLaren et al. 2020). Furthermore, the application of herbicides is prohibitively expensive in developing countries and not always feasible in vegetable and floriculture production systems (Kristiansen 2003, Martins et al. 2019, Chauhan 2020). Manual weeding is still widely practiced in many production systems worldwide, although high-labor costs reduce profits and economic sustainability. These limitations have led to efforts to develop alternative, environmentally friendly weed management options to reduce herbicide input while being suitable for organic and small-scale production.

Anaerobic soil disinfestation (ASD) is a pre-plant soil modification process that has been effective for the management of soilborne diseases and insect pests, with multiple benefits to alternative

farming systems. In ASD, soil is amended with readily decomposable carbon sources, then irrigated to saturation, and covered with plastic sheeting for 3 to 5 wk to create anaerobic conditions. An ancillary benefit observed in the research on ASD for plant disease suppression has been substantial suppression of weeds. The objectives of this review are to synthesize and evaluate the available literature on ASD from the perspective of weed management.

Anaerobic soil disinfestation was developed at the end of the 20th century independently in Japan (Shinmura 2000, Momma et al. 2013) and the Netherlands (Blok et al. 2000). The technique is also known as reductive soil disinfestation (RSD) in the Netherlands, as biological soil disinfestation in Japan, and bio-solarization in other areas of the world (Momma et al. 2013). The term bio-solarization indicates the combined effect of ASD and solarization, in which the use of transparent plastic is encouraged to trap solar heat instead of a light-impermeable tarp. Anaerobic soil disinfestation contains elements of soil solarization but may be more broadly applicable than soil solarization because ASD does not require high ambient temperatures or long periods of incubation (Momma et al. 2010). Furthermore, it builds up the organic matter content of the soil, causing beneficial shifts in soil microbial communities (Mazzola et al. 2018, Testen and Miller 2018, Tan et al. 2019), and enhances soil suppressiveness for diseases that might persist for long periods of time (Liu et al. 2019).

The addition of organic amendments to the soil to suppress soilborne diseases (Pugliese et al. 2015) and weeds (Ozores-Hampton et al. 2012) has been practiced in conventional and organic production systems for many years. The addition of organic matter under aerobic conditions enhances the production of volatile organic acids, which reduce seed viability and increase soil microbial activity leading to decay of weed seeds. Furthermore, amendment of organic matter under aerobic conditions also improves soil physiochemical properties and increases soil nutrient availability, which eventually strengthens plant health and ability to compete with weeds (Jiang et al. 2019, Liu et al. 2020a, Fujita et al. 2020).

Anaerobic soil disinfestation is more effective in suppressing weeds than the use of organic amendments under aerobic conditions because 1) soil saturation enhances the decomposition of organic matter, 2) anaerobic conditions foster the accumulation of toxic volatile fatty acids and other organic acids in amended soil (Greenwood 1961), 3) lack of oxygen suppresses weed seed respiration, and 4) anaerobic conditions result in changes in soil temperature and pH, which work synergistically with other factors to kill weed propagules.

Long-term weed management requires some means to reduce weed seed survival in the soil seedbank while also eliminating further seed input into soil. It is rarely possible to deplete the weed seed bank in soil by elimination of weed seeds through germination, predation, and death. Soil microbial attack and fluctuations in soil temperature and moisture enhance the breaking of seed dormancy, making seeds more susceptible to ASD treatment. Anaerobic soil disinfestation itself can stimulate seed germination, which is then followed by seed decay and disintegration.

## Alternative Approaches to Weed Management

There is a debate about the need to reorient concepts for dealing with weeds (MacLaren et al. 2020). An integrated approach that works to address several issues together could be sustainable, economically, and environmentally feasible. Such an approach could alter conventional practices such as crop rotation and cover cropping as well as identifying tools such as new biocontrol agents and bioherbicides or combinations of bioherbicide with other environmentally acceptable disinfestation techniques (Shrestha et al. 2016b) for a sustainable solution for weed management. Anaerobic soil disinfestation is a practice that could be part of an integrated approach to weed suppression in conventional and organic production systems. Inputs for ASD can be locally resourced and have little known potential for adverse environmental or health impacts. Anaerobic soil disinfestation can have broad-spectrum effects in farming systems for soilborne pathogen and insect pest management and positive impacts on crop productivity through quick turnover of soil nutrients and organic matter (Fujita et al. 2020). Reports of ASD effectiveness come from a range of environments, including the tropical high temperature region of Florida (above 30°C) (Muramoto et al. 2008) and low temperature region of Washington (16–26°C) (Hewavitharana and Mazzola 2020), where ASD was successful in reducing weed population density (Table 1).

Results of ASD studies have varied among weed species, soil types, incubation periods, temperatures, and types and forms of carbon source inputs applied (reviewed by Shrestha et al. 2016a). For example, ASD with rice bran (20 Mg ha<sup>-1</sup>) plus mustard seed meal (3.3 Mg ha<sup>-1</sup>) moderately reduced weed density in strawberry fields in California in 1 of 2 years of testing, while ASD with rice bran alone as the carbon source was ineffective both years compared

to steam and chemical fumigant treatments (Shennan et al. 2018). However, ASD was 85% effective for weed density suppression in tropical hot climates when molasses was used as an ASD carbon source in multi-location and multiyear trials (Di Gioia et al. 2016). These researchers observed yellow nutsedge (*Cyperus esculentus*) suppression in one of two experiments. Studies done in Tennessee showed significantly lower sprouting and reproduction of yellow nutsedge tubers when wheat bran but not dry molasses was used as the ASD carbon source under controlled and field experiments (Shrestha et al. 2018). Complete inhibition of yellow nutsedge tubers was observed with different carbon sources (wheat bran, molasses, ethanol, and broiler litter) in Florida when the tubers were buried at 15 cm depth in pots (Muramoto et al. 2008). The form of the carbon source is also an important factor to be considered in carbon source selection in terms of ease of application and efficacy. For example, Momma et al. (2013) reported that liquid forms of carbon sources such as ethanol are easier to apply in the field and penetrate the soil more thoroughly compared to solid forms of carbon sources such as wheat bran. A meta-analysis done by Shrestha et al. (2016a) reported higher efficacy (97%) of liquid forms of carbon sources compared to solid forms (44%) in weed suppression. More research is warranted to analyze the full impact of ASD on weed populations and seed bank dynamics and to identify effective carbon source inputs and rates and incubation periods according to local needs.

## Examples of ASD Impacts on Important Weeds

### Common Chickweed [*Stellaria media* (L.) Vill.] (Eudicots: Caryophyllaceae)

This annual weed species is native to Europe but is now considered one of the most widely distributed weeds throughout the world. Chickweed is one of the most sensitive weed species to ASD. The germination of chickweed seed was reduced by 60–74% when coffee grounds, paper mulch, peanut shells, rice bran, or brewers' spent grain were used as carbon sources in ASD compared to nonamended controls (Liu et al. 2020a).

### Barnyardgrass [*Echinochloa crus-galli* (L.) (Cyperales: Poaceae)

This annual grass species is widely distributed throughout the world, especially in warm and moist environments. It was among several grass and broadleaf weed species in eggplant and okra suppressed by ASD with various locally available carbon sources in Nepal (Khadka et al. 2020). Resident barnyardgrass seed germination was suppressed by 87–100% in ASD-treated soil amended with molasses, chicken manure, or wheat bran compared to the nonamended aerobic control (Khadka 2021). Similarly, ASD with mustard greens dry biomass at least 10 g kg<sup>-1</sup> soil caused 100% mortality of resident barnyardgrass seeds in a pot experiment in Ohio (Fig. 1).

### Common Lambsquarters [*Chenopodium album* (L.) (Caryophyllales: Amaranthaceae)

Common lambsquarters is a summer annual member of the goose-foot family (Chenopodiaceae). Due to its rapid growth rate and fast germination, it is a significant problem in many crops. It is less affected by ASD than other weed species, requiring mustard greens biomass of more than 10 g kg<sup>-1</sup> soil (equivalent to 20 mt ha<sup>-1</sup>) to achieve 100% loss of seed viability in growth chamber experiments. Results of Ohio field experiments depended on carbon source, with

**Table 1.** Summary of published studies on efficacy of anaerobic soil disinfestation (ASD) with different carbon sources in weed species suppression in both field and pot studies

Weed species	Location	Carbon source and application rate <sup>a</sup>	Duration	Reference
Annual bluegrass ( <i>Poa annua</i> ) Common purslane ( <i>Portulaca oleracea</i> )	Florida, USA* California, USA*	Wheat bran (10 Mg ha <sup>-1</sup> )	3 weeks	Muramoto et al. (2008)
Yellow nutsedge ( <i>Cyperus esculentus</i> )	Florida, USA*	Wheat bran (10 Mg ha <sup>-1</sup> ) Molasses (10 Mg ha <sup>-1</sup> ) Molasses (20 Mg ha <sup>-1</sup> ) Ethanol (1%, 100 mm) Broiler litter (20 Mg ha <sup>-1</sup> ) Broiler litter (20 Mg ha <sup>-1</sup> ) + molasses (10 Mg ha <sup>-1</sup> ) Broiler litter (20 Mg ha <sup>-1</sup> ) + molasses (20 Mg ha <sup>-1</sup> ) Broiler litter (20 Mg ha <sup>-1</sup> ) + ethanol (1%, 100 mm) Broiler litter (20 Mg ha <sup>-1</sup> ) + molasses (20 Mg ha <sup>-1</sup> ) + ethanol (1%, 100 mm)	3 weeks	Muramoto et al. (2008)
Slender amaranth ( <i>Amaranthus viridis</i> ) Bristly foxtail ( <i>Setaria verticillata</i> ) Common purslane ( <i>Portulaca oleracea</i> )	Turkey*	Olive processing waste (30 Mg ha <sup>-1</sup> ) Chicken manure (10 Mg ha <sup>-1</sup> )	6–7 weeks	Boz (2009)
Yellow nutsedge ( <i>Cyperus esculentus</i> ) Common purslane ( <i>Portulaca oleracea</i> )	Florida, USA* Spain <sup>†</sup>	Molasses (8.2 Mg ha <sup>-1</sup> ) Fresh chicken manure (12.5 Mg ha <sup>-1</sup> )	3 weeks	Butler et al. (2012a) Domínguez et al. (2014)
Annual bluegrass ( <i>Poa annua</i> ) Wimmera Ryegrass ( <i>Lolium rigidum</i> )				
Yellow nutsedge ( <i>Cyperus esculentus</i> ) Goosegrass ( <i>Eleusine indica</i> ) Southern Crabgrass ( <i>Digitaria ciliaris</i> ) Large crabgrass ( <i>Digitaria sanguinalis</i> ) Smooth crabgrass ( <i>Digitaria ischaemum</i> ) Pigweed ( <i>Amaranthus retroflexus</i> ) False daisy ( <i>Eclipta prostrata</i> ) Old world diamond ( <i>Hedyotis corymbosa</i> )	Florida, USA <sup>†</sup>	Molasses (22 Mg ha <sup>-1</sup> ) Composted poultry litter (22 Mg ha <sup>-1</sup> )	3 weeks	Di Gioia et al. (2016)
Yellow nutsedge ( <i>Cyperus esculentus</i> ) purple nutsedge ( <i>Cyperus rotundus</i> ) Different grass species Different broadleaf weeds	Florida, USA <sup>†</sup>	Molasses (6.9 m <sup>3</sup> ha <sup>-1</sup> ) Composted poultry litter (11 Mg ha <sup>-1</sup> )		Guo et al. (2017)
Creeping woodsorrel ( <i>Oxalis corniculata</i> ) Purple nutsedge ( <i>Cyperus rotundus</i> ) Bristly foxtail ( <i>Setaria verticillata</i> ) Nettleleaf goosefoot ( <i>Chenopodium murale</i> )	Spain <sup>†</sup>	Molasses (22 Mg ha <sup>-1</sup> ) Fresh goat manure (45 Mg ha <sup>-1</sup> )		Díaz-Hernández et al. (2017)
Black mustard ( <i>Brassica nigra</i> ) Black nightshade ( <i>Solanum nigrum</i> )	California, USA <sup>†</sup>	Mature green waste compost (2%) + tomato processing residues (2–5%)	8 days	Achmon et al. (2017)
Different weed species found in California	California, USA <sup>†</sup>	Rice bran (16.7 Mg ha <sup>-1</sup> )	3–6 weeks	Shennan et al. (2018)
Broadleaf thistles (Asteraceae) Mustards ( <i>Brassica</i> spp.) Fiddleneck ( <i>Amsinckia</i> spp.) Amaranthus ( <i>Amaranthus</i> spp.) Morning glory ( <i>Ipomoea</i> spp.) Caltrop ( <i>Tribulus terrestris</i> )	California, USA <sup>†</sup>	Mature green waste compost (2%) + tomato pomace mace (2.5%)	8 days	Achmon et al. (2018)

Table 1. Continued

Weed species	Location	Carbon source and application rate <sup>d</sup>	Duration	Reference
Yellow nutsedge ( <i>Cyperus esculentus</i> )	Tennessee, USA*	Wheat bran (3.2 to 7.8 g kg <sup>-1</sup> soil) + soybean meal (0 to 1.8 g kg <sup>-1</sup> soil)	3 weeks	Shrestha et al. (2018)
<i>Eclipta</i> sp. <i>Echinochloa</i> spp. <i>Panicum</i> spp. <i>Rumex obtusifolius</i> <i>Euphorbia hirta</i> <i>Sonchus</i> sp.	Nepal†	Wheat bran (3.2 to 7.8 g kg <sup>-1</sup> soil) + maize starch (0 to 6.7 g kg <sup>-1</sup> soil) Molasses (20 Mg ha <sup>-1</sup> ) Rice bran (20 Mg ha <sup>-1</sup> ) Raw goat manure (20 Mg ha <sup>-1</sup> ) Mustard cake (15 Mg ha <sup>-1</sup> ) Lentil ( <i>Lens esculenta</i> ) husks (20 Mg ha <sup>-1</sup> ) Berseem clover ( <i>Trifolium alexandrinum</i> ) leaves 30 Mg ha <sup>-1</sup> )	3 weeks	Khadka et al. (2020)
Little mallow ( <i>Malva parviflora</i> ) Annual sow thistle ( <i>Sonchus oleracea</i> ) Nettle leaf goosefoot ( <i>Chenopodium murale</i> ) Common purslane ( <i>Portulaca oleracea</i> ) Bristly oxtongue ( <i>Picris echioides</i> ) Sharppoint fluvellin ( <i>Kickxia elatine</i> ) Curly dock ( <i>Rumex crispus</i> ) Black nightshade ( <i>Solanum nigrum</i> ) Purple crabgrass ( <i>Digitaria sanguinalis</i> )	California, USA†	Sudangrass biomass (9.8 Mg ha <sup>-1</sup> )	5 weeks	Jacobs (2019)
Common chickweed ( <i>Stellaria media</i> ) Redroot pigweed ( <i>Amaranthus retroflexus</i> ) Yellow nutsedge ( <i>Cyperus esculentus</i> )	Virginia, USA*	Dry cowpea cover crop residue (10 g kg <sup>-1</sup> soil) Dry velvet bean cover crop residue (8.2 g kg <sup>-1</sup> soil) Dry buckwheat cover crop residue (11.7 g kg <sup>-1</sup> soil) Dry sorghum-sudangrass cover crop residue (9.8 g kg <sup>-1</sup> soil) Waste coffee grounds (16.5 kg <sup>-1</sup> soil) ± yeast (8.8 mg kg <sup>-1</sup> soil), Paper mulch (4.7 g kg <sup>-1</sup> soil) ± yeast (8.8 mg kg <sup>-1</sup> soil), Brewer's spent grain (9.4 g kg <sup>-1</sup> soil) ± yeast (8.8 mg kg <sup>-1</sup> soil) Rice bran (9.4 g kg <sup>-1</sup> soil) ± yeast (8.8 mg kg <sup>-1</sup> soil), Peanut shell (9.2 g kg <sup>-1</sup> soil) ± yeast (8.8 mg kg <sup>-1</sup> soil)	3 weeks	Liu et al. (2020a)
Common dandelion ( <i>Taraxacum officinale</i> ) Tall tumble mustard ( <i>Sisymbrium altissimum</i> ) Annual meadow grass ( <i>Poa annua</i> ) Shepherd's purse ( <i>Capsella bursa-pastoris</i> ) Henbit deadnettle ( <i>Lamium amplexicaule</i> ) Hairy crabgrass ( <i>Digitaria sanguinalis</i> ) Jagged chickweed ( <i>Holosteum umbellatum</i> ) <i>Polygonum</i> spp. Common mallow ( <i>Malva neglecta</i> )	Washington, USA†	Orchard grass ( <i>Dactylis glomerata</i> ) biomass (dry, 20 Mg ha <sup>-1</sup> )	2 weeks	Hewavitharana and Mazzola (2020)

Table 1. Continued

Weed species	Location	Carbon source and application rate <sup>a</sup>	Duration	Reference
Redroot pigweed ( <i>Amaranthus retroflexus</i> )	Ohio, USA*	Chicken manure (10.1, 20.2, 40.4 and 74 Mg ha <sup>-1</sup> )	3–4 weeks	Khadka (2021)
Common lambsquarters ( <i>Chenopodium album</i> )		Molasses (10.1, 20, 40.2 and 74 Mg ha <sup>-1</sup> )		
Yellow nutsedge ( <i>Cyperus esculentus</i> )		Mustard greens biomass (dry, 10.1, 20.2, and 40.4 Mg ha <sup>-1</sup> )		
Common pokeweed ( <i>Phytolacca decandra</i> )		Wheat bran (10.1, 20.2, 30.3 and 74 Mg ha <sup>-1</sup> )		
Black nightshade ( <i>Solanum nigrum</i> )		Wheat bran (10.1, 20.2, 30.3 and 74 Mg ha <sup>-1</sup> )		
Dandelion ( <i>Taraxacum officinale</i> )		Wheat bran (10.1, 20.2, 30.3 and 74 Mg ha <sup>-1</sup> )		
Barnyardgrass ( <i>Echinochloa crus-galli</i> )		Wheat bran (20.2 Mg ha <sup>-1</sup> ) plus molasses (10.1 Mg ha <sup>-1</sup> )		

<sup>a</sup>Values inside the parentheses are application rates of carbon sources.

\*Greenhouse and growth chamber studies.

†Field studies.

46% seed mortality when molasses was the carbon source and 57% with wheat bran plus molasses, compared to 8% in the aerobic control (Khadka 2021).

#### Black Nightshade [*Solanum nigrum* (L.)] (Solanales: Solanaceae)

This annual weed in the nightshade family (Solanaceae) has been shown to be sensitive to ASD treatment. Anaerobic soil disinfestation with 2% mature green waste and 2–5% tomato processing residues resulted in more than 95% inactivation of *Solanum nigrum* seeds (Achmon et al. 2017). Similarly, the mortality of *S. nigrum* seeds was 77% in molasses-amended and 82% in wheat bran plus molasses-amended ASD-treated soils in field experiments in Ohio (Khadka 2021).

#### Yellow Nutsedge [*Cyperus esculentus* (L.)] (Poales: Cyperaceae)

This important perennial is cosmopolitan in distribution and reproduces by seeds and tubers. This weed species is particularly challenging in polyethylene-mulched production systems, as leaves can perforate opaque mulch due to their sharp tips (Daugovish and Mochizuki 2010). Multiple studies have shown that nutsedge tubers are quite sensitive to ASD. For instance, ASD with paper mulch, peanut shells, and rice bran used as carbon sources caused 100% tuber mortality (Liu et al. 2020a). Similarly, significantly lower tuber sprouting was observed in ASD-treated soil compared to the nonamended control regardless of the carbon source used (Muramoto et al. 2008, Shrestha et al. 2018).

#### Redroot Pigweed [*Amaranthus retroflexus* (L.)] (Caryophyllales: Amaranthaceae)

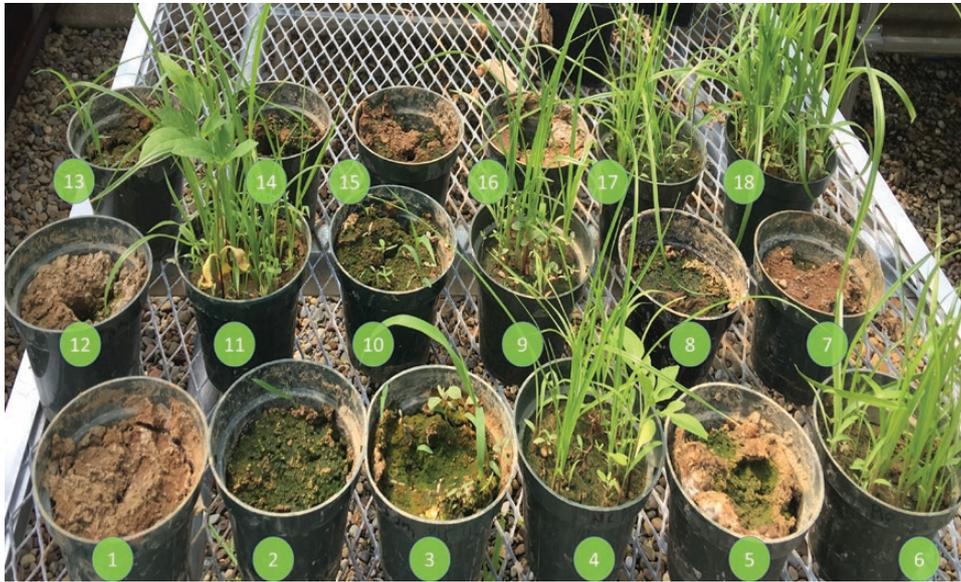
Redroot pigweed is a common summer annual that is distributed worldwide. It is one of the weed species with moderate sensitivity to ASD treatment. Liu et al. (2020a) reported 61–76% suppression of seed germination relative to the nonamended control when exposed to ASD with brewers' spent grain, coffee grounds, paper mulch, peanut shells, or rice bran carbon sources. Similarly, significantly greater seed mortality of redroot pigweed was observed when buckwheat, cowpea, and velvet bean biomass were used as ASD

carbon sources compared to nontreated controls (Liu et al. 2020b). Anaerobic soil disinfestation with more than 20 Mg ha<sup>-1</sup> mustard greens biomass resulted in 80% germination inhibition of resident seeds in growth chamber experiments (Khadka 2021). Mortality of pigweed seeds was 46% in soil amended with molasses and 54% in soil amended with wheat bran plus molasses then subjected to ASD under field conditions in Ohio (Khadka 2021).

#### Other Weed Species

White clover [*Trifolium repens* (L.)] (Fabales: Fabaceae) seed viability was reduced by 50–100% relative to the nontreated control when rice bran, paper mulch, brewer's spent grain, waste coffee grounds, peanut shells or biomass of sorghum-sudangrass, cowpea, or buckwheat cover crops were applied at 4 mg of C/g soil as the ASD carbon source under greenhouse conditions in Virginia (Liu et al. 2020a). Overall weed biomass was significantly reduced in okra and eggplant fields after ASD with molasses, rice bran, rice bran plus molasses, raw goat manure, mustard (*Brassica campestris* var. *toria*) cake, lentil (*Lens esculenta*) husks, or berseem clover (*Trifolium alexandrinum*) leaves compared to a nonamended, aerobic control in Nepal (Khadka et al. 2020). Reductions of 100% were observed in weed populations of slender amaranth [*Amaranthus viridis* (L.)] (Caryophyllales; Amaranthaceae), bristly foxtail [*Setaria verticillata* (L.)] (Poales: Poaceae), and common purslane [*Portulaca oleracea* (L.)] (Caryophyllales: Portulacaceae) when olive processing waste (30 Mt ha<sup>-1</sup>) or chicken manure (10 Mt ha<sup>-1</sup>) was applied with a biosolarization system very similar to ASD in Turkey (Boz 2009; Table 1).

Weed density was reduced by 88% at 45 d and 51% at 165 d after transplanting tomatoes compared to noncovered and nonamended control plots when goat manure was used as the carbon source in bio-solarization (Díaz-Hernández et al. 2017). The predominant weed species in these experiments were creeping woodsorrel (*Oxalis corniculata*), purple nutsedge (*Cyperus rotundus*), bristly foxtail (*Setaria verticillata*), and nettleleaf goosefoot (*Chenopodium murale*). Achmon et al. (2018) analyzed the effect of biosolarization with tomato pomace or mature green waste compost on weed seed mortality at different soil depths. They found significantly lower weed density and germination at both soil depths tested (7.5–15 and 15–22.5 cm) in bio-solarized plots compared to solarized plots. The predominant weed species in these experiments were cool-season



**Fig. 1.** Effect of anaerobic soil disinfestation (ASD) with different rates of dry mustard greens biomass amendments on resident weed seed germination. Amendment rates  $\text{kg}^{-1}$  soil: 20 g (1,7,15), 15 g (2,8,14), 10 g (5,12,16), 5 g (3,10,13), 0 g (4,9,17; nonamended covered/anaerobic controls) and (6,11,18; nonamended uncovered/aerobic controls). The numbers in the parentheses indicate pot number. The predominant weed species were barnyardgrass, redroot pigweed, and common lambsquarters. Pot experiments were conducted by mixing different amounts of dry mustard greens biomass with soil collected from an organic farm. Pots were by flooded and double sealed in Ziploc bags and incubated in a growth chamber for 3 wk. Then the pots were maintained in a greenhouse to monitor the germination of resident weed seeds.

monocots (Poaceae), broadleaf thistles (Asteraceae), mustards (Brassicaceae), fiddleneck (Boraginaceae), warm-season monocots (Poaceae, Cyperaceae), broadleaf thistles (Asteraceae), amaranths (Amaranthaceae), morning glory (Convolvulaceae), and caltrop (Zygophyllaceae).

Domínguez et al. (2014) reported that during 10 yr of experimentation with biosolarization with fresh chicken manure in strawberry production, there was year-by-year improvement in suppression of common purslane (*Portulaca oleracea*), annual bluegrass (*Poa annua*), and Wimmera ryegrass (*Lolium rigidum*) compared to standard chemical fumigants. A field study from Spain documented a significant reduction in emergence of weeds dominated by creeping woodsorrel [*Oxalis corniculata* (L.)] (Oxalidales: Oxalidaceae), purple nutsedge [*Cyperus rotundus* (L.)] (Poales; Cyperaceae), bristly foxtail [*Setaria verticillata* (L.)] (Poales: Poaceae), and nettleleaf goosefoot [*Chenopodium murale* (Fuentes, & Borsch) (Caryophyllales: Amaranthaceae) in biosolarized plots with fresh goat manure compared to solarized, chemically (metam sodium) fumigated, and nontreated control plots (Díaz-Hernández et al. 2017).

## Potential Mechanism of Action of ASD Against Weeds

### Creation of Stale Seedbed

Soil disturbance during incorporation of carbon sources into the soil exposes weed seeds to the light and air, and irrigation provides the required moisture, eventually stimulating weed seed germination. Furthermore, initial temperature increases due to solarization where applicable, i.e., when a transparent plastic tarp is used, provide an excellent environment for weed seed germination. When carbon sources start to break down, the environment drastically changes and becomes unfavorable for juvenile weed seedlings. Use of transparent plastic in ASD might encourage weed seed germination at the beginning of the process since almost 50% of weed species require light for seed germination (Andersson et al. 1997, Riemens et al.

2007). Therefore, reducing weed seed populations through germination could be one of the important mechanisms in ASD.

### Poorly Oxidized Metabolites

The ASD process involves three distinct phases: aerobic, facultatively anaerobic, and anaerobic. During facultatively anaerobic and anaerobic phases of ASD, free oxygen declines in the soil. Therefore, soil microbes utilize nitrate, iron, manganese oxides, sulfate, and carbon dioxide as electron acceptors during carbon source degradation (respiration; Staley and Orians 2000). Anaerobic breakdown of carbon sources produces poorly oxidized compounds such as methane and ethylene gases, alcohol, hydrogen sulfide, and organic acids (Gao et al. 2004), which are toxic to weed seeds. Hewavitharana et al. (2014) reported that additional biocidal compounds such as dimethyl trisulfide, 2-ethyl-1-hexanol, decanal, and nonanal were produced during decomposition of carbon sources during ASD. Increased production of hydrogen sulfide also has been reported under anaerobic conditions in soil. Free hydrogen sulfide is toxic to many plant species, including rice, but toxicity effects are quickly recovered from once oxygen becomes available (Gao et al. 2004). Volatile fatty acids produced during ASD are considered to be major factors contributing to weed seed mortality (Achmon et al. 2017; Liu et al. 2020a).

Acetic acid concentrations over  $>30 \text{ mg kg}^{-1}$  are believed to be phytotoxic to plants (Shiralipour and McConnell 1991). Anaerobic soil disinfestation has been shown to generate organic acids in concentrations high enough to kill weed seeds. Total organic acids from 1283 to 4718  $\text{mg kg}^{-1}$  including acetic acid (1046 to 1442  $\text{mg kg}^{-1}$ ), propionic acid (122 to 333  $\text{mg kg}^{-1}$ ), isobutyric acid (59 to 308  $\text{mg kg}^{-1}$ ), and butyric acid (419 to 2729  $\text{mg kg}^{-1}$ ) in soil were reported when mustard greens, ethanol, wheat bran, or molasses were used as carbon sources in ASD (Sanabria-Velazquez et al. 2020). In another study, acetic acid amounts ranging from 250 to 500  $\text{mg kg}^{-1}$  and propionic acid between 300  $\mu\text{g g}^{-1}$  and 350  $\mu\text{g g}^{-1}$  were detected when green waste and green waste plus compost were used as carbon sources in ASD (Hestmark et al. 2019).

## Anaerobic Conditions

The presence of hypoxic conditions for imbibed seeds leads to the loss of viability through the process known as soaking injury (Crawford 1977). Anaerobic soil disinfestation maximizes soaking injury by providing sufficient moisture for seed imbibition initially, followed by anaerobic conditions. During seed germination, ethanol is produced internally due to the anaerobic breakdown of glucose and needs to be eliminated quickly from seeds. However, this does not happen in an anaerobic environment during ASD, which leads to the self-poisoning effects of ethanol in soil. Redox reactions occurring during ASD further exacerbate the toxic environment by accumulating ethylene gases, alcohol, and organic acids. The environmental conditions in soil during ASD are similar to those encountered by deeply buried seeds where viability is lost due to endogenous production of gas inhibitors during the metabolic fermentation of seed (Benvenuti and MacChia 1995). Holm (1972) identified the metabolites acetaldehyde, acetone, and ethanol as responsible for loss of weed seed viability in deep soil profile. Production of these metabolites is induced by low oxygen conditions in the soil.

## Effects of pH Changes

Anaerobic soil disinfestation treatment tends to change the soil pH depending upon the soil type. Generally, an accumulation of short-chain fatty acids such as acetic and butyric acids is likely to decrease the soil pH (Momma et al. 2006). Soil-reducing conditions followed by reduced soil pH under anaerobic conditions are likely to increase the accumulation of heavy metal ions such as  $\text{Fe}^{2+}$ ,  $\text{Mn}^{2+}$  (Momma et al. 2011), and  $\text{Al}^{3+}$ , which have direct toxicity to weeds seeds and seed juveniles (Ma et al. 2017). Furthermore, the accumulation of toxic metals such as aluminum in acidic soil leads to reduced seed bank viability in soil (Pakeman et al. 2012). However, soil pH reduction does not always occur during ASD. For example, redox reactions under anaerobic conditions increase soil pH and the soil profile may change from acidophilic to alkaliphilic. Significant correlations between concentrations of toxic metals and viability of weed seeds during the manure decomposition process have been reported (Liu et al. 2020b). However, direct effects of soil pH fluctuation and metal ion concentration on weed seed mortality during ASD still need to be studied.

## Elevated Temperature

Anaerobic soil disinfestation treatments increase soil temperatures due to two likely reasons: exothermic reactions during carbon source breakdown and the solarization effects of plastic tarps. The soil temperature raised by ASD might not be sufficient to kill weed seeds; however, the imbibed seeds and juvenile seedlings are more sensitive to temperature change in soil. Furthermore, high temperature might work synergistically with other factors such as soaking injury and poorly oxidized metabolites produced during ASD. Achmon et al. (2017) reported higher weed seed mortality while combining solar heating with organic matter than either alone.

## Metal Ions

Redox reactions during ASD lead to the accumulation of metal ions such as  $\text{Fe}^{2+}$  and  $\text{Mn}^{2+}$  in soil. Reduction of  $\text{Mn}^{2+}$  and  $\text{Fe}^{2+}$  oxyhydroxides to produce  $\text{Fe}^{2+}$  and  $\text{Mn}^{2+}$  by adding rice straw under anaerobic conditions created by continuous flooding has also been reported (Gao et al. 2004). The highest concentration of  $\text{Fe}^{2+}$  and  $\text{Mn}^{2+}$  in soil solution was reached 3 to 6 wk after flooding depending on the

amount of rice straw added, with maximum concentrations higher and occurring earlier at higher rice straw amounts. Fungicidal, nematocidal, and phytotoxic effects of increased  $\text{Fe}^{2+}$  and  $\text{Mn}^{2+}$  have been reported (Momma et al. 2011). Soil microbes such as *Desulfosporosinus* and *Bacillus* produce sulfide and low-valence metal ions ( $\text{Fe}^{2+}$ ,  $\text{Mn}^{2+}$ ) (Boone et al. 1995, Stanley and Southam 2018), respectively, which are toxic to imbibed weed seeds and seed juveniles.

## Microbial Degradation

Weed seed longevity in the soil is largely influenced by the activities of the soil microbial community. Soils having high biological activity are more suppressive to weeds than those that do not (Nikolić et al. 2020). Several layers of soil microbiota change over time during the ASD process. Anaerobic soil disinfestation provides opportunities for a variety of microbes to damage imbibed weed seeds through three distinct microbial phases: 1) aerobic, 2) facultatively anaerobic, and 3) anaerobic. These phases are distinguished based on the soil physiochemical and microbial composition (Hewavitharana et al. 2019). During the aerobic phase, oxygen concentrations remain high so carbon source degradation is mostly dominated by aerobic microbes such as *Streptomyces* spp., *Bacillus* spp. and *Pseudomonas* spp., *Pasteuria* spp., *Chloridium* spp., and *Trichoderma* spp. Due to rapid multiplication of aerobic microbes, the oxygen availability in the soil environment declines and the dominance of facultative anaerobic microbes is established. Microbial communities during this phase are mostly dominated by Firmicutes, Actinobacteria, Proteobacteria, and Zygomycota in facultative anaerobic phase. Oxygen concentration is further reduced, and carbon dioxide and hydrogen sulfide concentrations are increased in the soil. This phase is also characterized by the formation of metabolites such as lactic, butyric, and acetic acids and acetone butanol. In the anaerobic phase, free oxygen is no longer available and microbes that can utilize oxygen from metal oxides become dominant. This phase is characterized by the presence of strictly anaerobic microbes such as *Clostridium* spp. and the accumulation of lipids, glycerol, organic acids, aldehydes, and alcohols. Furthermore, accumulation of dimethyl disulfide (DMS), dimethyl trisulfide (DMTS), and dimethyl sulfoxide in soil has also been reported (Hewavitharana et al. 2019). The length of each phase depends on the nature of carbon source, soil temperature, and physiochemical conditions. Furthermore, ASD increases organic matter content in soil; increased organic matter directly correlates with weed seed suppression and higher weed seed decay (Cardina et al. 1991, Davis et al. 2006).

## Multiple Benefits in Farming Systems

Anaerobic soil disinfestation increases soil fertility by enhancing soil organic matter content and nutrient turnover (Martínez-García et al. 2018). It also helps us to rehabilitate soil degraded by overuse of agrochemicals, salinization, and pathogen infestation (Zhu et al. 2014). Anaerobic soil disinfestation conditions soil pH by bringing it towards neutral from alkaline or acidic conditions (McCarty et al. 2014, Zhu et al. 2014). It enhances the soil's resilience by increasing buffering capacity through the addition of organic matter, soil pH balance, and increased soil microbial activity (Liu et al., 2019). The efficacy of ASD in suppressing soilborne pathogens, nematodes, and insect pests has been widely reported (Momma et al. 2010, Khadka et al. 2020, Liu et al. 2019, Sanabria-Velazquez et al. 2019, Testen and Miller 2019, Khadka and Miller 2021). These multiple contributions to soil health enhance crop competitiveness with weeds and promote crop productivity.

## Application to Smallholder Farming

Weed management practices are quite different in smallholder agriculture than in large-scale mechanized systems. Synthetic herbicides, chemical fumigants, flaming, steaming, or other weed management tools are not accessible or prohibitively expensive in lower resourced farming. Most growers in less industrialized countries use intensive tillage as a tool to manage weeds; however, intensive tillage can result in loss of organic matter and soil moisture, soil quality degradation, and reduced profits due to high labor costs (Kubitza et al. 2020). Crop losses due to poor weed management practices can be significant, threatening food security in less industrialized countries (Ali et al. 2017). Weed management on smallholder farms in these countries is usually based on hand-weeding, often carried out by children and women (Chauhan 2020, Brown et al. 2021). Use of ASD on smallholder farms could reduce the need for hand-weeding while improving soil health as described above.

Carbon sources needed for ASD can be locally resourced in countries dominated by smallholder agriculture. The only nonrenewable resource needed is plastic, which is generally available and can be reused. Any type of impermeable plastic available in local markets can be used for ASD. Clear plastic sheeting traps solar energy to enhance ASD efficacy, although opaque plastic is also effective. Smallholder farmers depend on organic matter such as composted animal manures and other composts for plant nutrients. These organic matter resources can be utilized as carbon sources in ASD. At the same time, there is also the potential to use fast-growing cover crop biomass (see below) where other carbon sources are limited.

## Integrating ASD With Cover Crops

Short season cover crops terminated before weed seed set can be utilized as ASD carbon sources. Cover crops can increase soil organic matter content and microbial activity and reduce nitrate leaching, could contribute nitrogen to subsequent crops, and enhance soil quality (Schipanski et al. 2014). Cover crops can also help us to suppress weed seed germination and inhibition of seedling growth by allelopathy (Mohler et al. 2012). Furthermore, the soil disturbance during cover crop sowing and management stimulates the germination of weed seeds and these weeds can be killed before seed set during cover crop termination for ASD. Incorporating sudangrass biomass into soil followed by solarization resulted in better weed control than solarization alone and sudangrass biomass mulching alone in strawberry production systems in California (Jacobs 2019). The weeds that were suppressed included little mallow [*Malva parviflora* (L.)] (Malvales; Malvaceae), annual sow thistle [*Sonchus oleraceus* (L.)] (Asterales; Asteraceae), nettle leaf goose-foot [*Chenopodium murale*], and common purslane (*Portulaca oleracea*) among others (Table 1).

## Variable Effects of ASD on Weed Suppression

The effectiveness of ASD in weed suppression varies across field conditions, weed species, carbon sources, and local environmental conditions (reviewed by Shrestha et al. 2016a). For instance, almost 100% mortality of black mustard [(*Brassica nigra* (L.)) (Asterales: Asteraceae) and black nightshade (*Solanum nigrum*) seeds was observed following biosolarization at higher temperature, but seed mortality was not reduced at room temperature compared to nonamended controls (Achmon et al. 2017). The authors speculated that higher temperature interacted with volatile and nonvolatile metabolites to induce mortality. Therefore, selecting appropriate

carbon sources might be more important at lower temperature conditions than higher temperatures, since production of these metabolites varied according to the carbon source inputs in ASD (Hewavitharana et al. 2019). In some studies, ASD with cover crop biomass alone as the carbon source did not suppress the germination of yellow nutsedge tubers, whereas addition of molasses reduced germination even though soil reducing conditions were similar between cover crops- and molasses-amended ASD-treated plots (Butler et al. 2012b). In other studies, ASD with grass residues reduced weed biomass at low temperatures (16 to 23°C) (Hewavitharana and Mazzola 2020). A field treatment with ASD-dry molasses + soybean meal with or without bioherbicides did not significantly reduce weed density in Tennessee compared to the nontreated control for both monocot and dicot weed species (Shrestha et al. 2016b).

The type of plastic used in ASD seems to have little effect on results. For example, Muramoto et al. (2008) evaluated the effects of different plastic tarps (white and black; both 1.25 mil standard polyethylene films), virtually impermeable film [1.25 mil embossed black tarp], or pit tarp [8 mil black/white tarp] and different soil types and temperatures on germination of annual bluegrass or common purslane after ASD. They reported no significant impact of tarp type, soil type, or temperature on mortality of the weed seeds tested. However, no germination of yellow nutsedge tubers was observed when tubers were buried at 15 cm compared to 66% germination at 2 cm depth, indicating a significant impact of propagule depth on weed seed mortality.

In some cases, ASD has failed to suppress weed seed germination or affect seed mortality. For example, ASD did not affect crabgrass [(*Digitaria sanguinalis* (L.)) (Poales: Poaceae) germination, and furthermore, it enhanced the germination of pigweed (reviewed by Shrestha et al. 2016a). Variation in weed response to ASD could be due to the high diversity among weed species under field conditions and differences in seed dormancy and sensitivity to physical and chemical factors such as heat and organic acids between species. Weed species with impermeable or hygroscopic or hard seed coats, such as pigweed, are potentially less sensitive to ASD because they do not imbibe water at the beginning of ASD. In some species, the presence of covering structures such as lemma and palea (e.g., crabgrass) could potentially prevent direct contact between ASD metabolites and seed embryos (Gallart et al. 2008), thereby decreasing ASD effects on seed mortality.

## Further Research Needs

Anaerobic soil disinfestation has been effective in suppressing weeds in numerous farming systems. However, more research is needed focusing on aspects of ASD such as sensitivity of different weed species, feasibility of locally available carbon sources and their interaction with soil types, duration of incubation, and environmental temperature requirements. Studies should determine how to optimize the use of cover crops as ASD carbon sources for suppression of different weed species and identify additives to carbon sources such as yeast or specific microbial consortia to increase the efficacy of ASD. Research is also needed to develop equipment, machines, or tools for laying plastic mulch and carbon source applications in the field at the optimal depth for wider adaptability in large-scale farming systems. Limited research indicates the long-term application of ASD could potentially produce greenhouse gases such as methane and hydrogen sulfide (Sanabria-Velazquez et al. 2020) and N<sub>2</sub>O (Zhao et al. 2021). Methane production is mostly affected by the type of carbon source applied, soil environment, and duration of ASD treatment (Sanabria-Velazquez et al. 2020). Anaerobic soil

disinfestation with straw and manure as carbon sources increased methane production compared to the nontreated control but methane production from ASD was negligible with regard to the Global Warming Potential (Zhao et al. 2021). However, N leaching in ASD-treated plots amended with manure due to N<sub>2</sub>O emission was enormously higher compared to the average emissions from global scale agriculture (Zhao et al. 2021). Therefore, Zhao et al. (2021) argued against using manure as an ASD carbon source. This indicates the necessity of further research on environmental impacts of ASD. Similarly, research to reduce methane production by amendment of methanogenesis inhibitors and their applicability to weed seed mortality could provide a new avenue of research to increase the efficacy of ASD for weed management.

Anaerobic soil disinfestation could reduce the need for herbicides, pesticides, and chemical fertilizers due to its multiple beneficial roles in crop production. However, increasing the use of nondegradable low-density polyethylene plastic mulches in agriculture has raised concerns about the sustainability of agroecosystems due to accumulation of plastic wastes. Alternatives to plastic currently used in ASD, such as bioplastic or biodegradable plastic mulch, need to be identified. Currently available biodegradable plastic films are only partially impermeable and would be unlikely to create strict anaerobic conditions needed for ASD. Advances in plastic recycling technology and recycled plastic product development could also lessen concerns regarding widespread adoption of another plastic-intensive crop management tool.

## Conclusions

Considering the challenges associated with current weed management approaches, ASD offers a new ecologically based weed management process that is suitable for organic as well as smallholder farming. Implementing ASD as a tool for weed management could provide a sustainable solution for multiple issues in farming systems. Integration of ASD with cover crops and conventional organic matter applications could reduce the initial investment for ASD treatments without compromising their individual benefits. Therefore, ASD could be an important tool for integrated pest management programs worldwide where labor costs are increasing rapidly and herbicides and large equipment use are not feasible or preferred for weed management. However, the success of ASD for weed suppression depends on soil temperature, carbon source, and soil microbes and their interactions with weed species. Therefore, some variability in weed suppression by ASD is expected depending on the local climate, soil characteristics, carbon source inputs, and resident weed species. Tactical use of ASD based on an understanding of local climate-edaphic factors, weed species composition, grower needs, and accessibility of carbon source inputs may be needed for long-term management of weeds along with soilborne disease suppression. It would be worthwhile to conduct more site-specific investigation on the impact of ASD components on a variety of weed species for broader applicability and greater adoption in multiple farming systems.

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