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Review

Bioherbicides in Organic Horticulture

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Abstract: Organic horticulture producers rank weeds as one of their most troublesome, time-consuming, and costly production problems. With the increasing significance of organic horticulture, the need for new bioherbicides to control weeds has grown. Potential bioherbicides may be developed from pathogens, natural products, and extracts of natural materials. Fungal and bacteria pathogens are two important types of microbial agents that have potential to be used as bioherbicides. The byproducts of natural sources such as dried distillers grains with solubles (DDGS), corn gluten meal (CGM), and mustard seed meals (MSMs) have shown herbicidal activities in controlling many weed species. Some essential oil extracts have shown bioherbicide potential as well. The efficacy of a bioherbicide is the main limiting factor for its application, and it may be affected by environmental factors such as humidity and moisture, the application method, the spectrum of the bioherbicide, and the type of formulation. In addition to efficacy, costs and concerns about potential human health threats are also limitations to bioherbicide use. As the integration of bioherbicide technology into current weed management systems may help manage herbicide resistance, reduce production costs, and increase crop yields, future research should involve the development of more cost-effective and efficient bioherbicides for control of weeds, as well as the optimization of production methods and cultural practices with use of candidate bioherbicides.

Keywords: bioherbicide; distillers grains with solubles; corn gluten meal; mustard seed meal; *essential oil*

1. The Problem of Weeds in Organic Horticulture

Weeds are the most costly category of agricultural pests, causing great yield loss and labor expense [1]. Agricultural weeds can emerge rapidly, resulting in reduction of crop plant growth and quality by competing for nutrients and water provided to crops and producing chemicals that suppress crop growth. Annual weeds reproduce through prolific seed production, and they germinate in responses to light, increased fluctuations in soil temperature and moisture, improved aeration, and accelerated nutrient release, while perennial weeds regenerate new plants from small fragments of roots, rhizomes, stolons, and other underground structures [1]. Severe weed problems present a serious threat to horticultural crop production with favorable environmental conditions, a susceptible crop, or a large weed seed bank in the soil.

Current weed control in horticultural production includes conventional herbicides (pre-emergent and post-emergent), organic herbicides, physical methods (hand-weeding and mulches), and bioherbicides. Pre-emergence herbicides are effective before and during weed seed germination. When germinating seeds are in contact with the herbicide, the growth of emerging roots and/or shoots is inhibited, but pre-emergent herbicides may not be effective without good contact with germinating weed seeds [2]. Post-emergent herbicides are effective after weeds have emerged from the soil, ideally at the seedling stage. Organic herbicides need to be applied either prior to crop seedling emergence or transplanting, or post-directed to established crop plantings assuring that the herbicides do not cause injury on the crop plants. Current organic herbicides include ammonium nonanoate, fatty acids, vinegar, clove oil, and D-limonene [3]. Broadcast application of vinegar and clove oil has been studied for potential use in weed management on young, actively growing sweet corn, onion, and potato [4]. Physical methods in weed control include hand-pulling and mulches (including weed discs), which is necessary with some high-value crops but it is labor intensive, time-consuming, and expensive [5]. In addition to the above methods, grazing by domestic goats has also resulted in significant control of many weed species [6]. Combining hand-weeding and spot treatment with post-emergent herbicides after pre-emergent herbicide application has provided complete weed control [5].

Organic horticulture is expanding worldwide, driven by consumer demand, resource conservation, and food security in North American and European markets [7]. Expanding organic production has implied production of nutritionally-improved food crops while using fewer external inputs and reducing environmental impacts [8]. Horticultural crops, especially fruits and vegetables, are critical components of a healthy diet. In some studies, organic foods contained more nutrients and vitamins compared to conventionally-produced ones, and have grown to play an important role in consumer purchases [9]. In 2013, the North American organic food and drink market was valued at 35 billion US dollars, and a healthy market growth rate was predicted [10]. Organic horticultural crops may be more difficult to grow than conventionally-produced crops due to organic production regulations governing the use of materials for control of insect, disease, and weed control challenges. With the high costs of pest and weed control, and the time, and labor in managing the system, organic horticulture relies on price premiums for economic viability, which may make it more profitable than conventional horticulture depending on management strengths and cultural practices.

Weed control in organic horticulture does not have simple or standard solutions. Organic farmers need to take long-term approaches to control weeds without causing yield loss. Successful organic weed control needs to begin with an ecological understanding of weeds and their roles in the farm or garden ecosystem [1]. In organic horticulture, hand-weeding and cultural methods should be integrated to prevent the occurrence of weed-induced yield losses and to keep down costs for weed control. Because organic horticulture excludes the uses of synthetic herbicides due to their potential contamination of crops and natural resources, the use of bioherbicides to control weeds through the use of natural products, extracts, and natural biological agents such as fungi and bacteria to attack weeds is becoming an effective tool [11].

2. Bioherbicide Approach

Biological controls have been developed for weed management using either living organisms, such as insects, nematodes, bacteria, or fungi, or natural products. Bioherbicides offer a sustainable, low cost, and environmentally-friendly approach to complement conventional methods, which helps meet the need for new weed management strategies. There are two main approaches to biological weed control: classical biological control and bioherbicide approach [12]. The classical biological approach introduces a natural enemy that spreads throughout the area where the target weed occurred [13]. However, this approach has the risk of attacking non-target plants after the introduction of the biocontrol agent in a new area [14]. The classical approach is subjected to strict regulations because of the introduction of potentially harmful pathogens to agricultural production. The bioherbicide approach relies on natural enemies present within the native range of the weed to cause significant damage to the weed and reduce the negative impact on crop yield [13]. The classical approach is based on the innate capacity of natural enemies to reproduce, while the bioherbicide approach is based on reproduction of natural enemies under controlled conditions and subsequent spread by man [13]. The bioherbicide approach is preferred over the classical approach, because it offers diverse possibilities for use in agricultural systems, lawns, and gardens. With the increasing importance of the role of bioherbicides in organic horticulture, the main objective of the following discussion is to review the effectiveness of various bioherbicide approaches.

2.1. Bioherbicides from Pathogens

There have been many microbial agents under evaluation for their potential as bioherbicides with horticultural crops, turf, and forest trees, including obligate fungal parasites, soil-borne fungal pathogens, non-phytopathogenic fungi, pathogenic and non-pathogenic bacteria, and nematodes [11]. One of the first bioherbicides registered was DeVine (Encore Technologies, Plymouth, MN, USA) with the active ingredient *Phytophthora palmivora*, which was developed to control strangler vine (*Morrenia odorata*) on citrus in Florida [15]. In the subsequent quarter century, several more pathogenic fungi and bacteria have been developed to control weeds [15]. Using plant pathogens as biocontrol agents can cause severe damage to target weed species. In order to become suitable pathogens, they must be mass-produced and their pathogenicity tested on weeds in a range of environmental conditions, followed by field efficacy and host range tests [16]. A variety of phytotoxins produced by plant pathogens can interfere with plant metabolism, ranging from subtle effects on gene expression to plant mortality [17].

Some fungal pathogens are toxic to a wide range of weed species. The early mycoherbicides ("DeVine", "Collego" with the active ingredient *Colletotrichum gloeosporioides* f. sp. *aeschynomene*, "Biomal" with the active ingredient *Colletotrichum gloeosporioides*) had highly virulent fungal plant pathogens that could be mass-cultured to produce large quantities of inoculum for inundative application to the weed host. These fungi infect the aerial portion of weed hosts, resulting in visible disease symptoms [11]. The rust fungus *Puccinia canaliculata* is a foliar pathogen of yellow nutsedge (*Cyperus esculentus*), and it can be mass-cultured on the weed host in small field plots or the greenhouse [18]. Applying the fungal pathogen *Chonrotereum purpureum* to wounded branches or stumps of weedy tree species inhibited re-sprouting and decayed the woody tissues [19]. Weidemann *et al*. (1992) [20] reported that the fungal pathogen *Microsphaeropsis amaranthi* controlled certain pigweed (*Amaranthus*) species, while *Phoma proboscis* controlled field bindweed (*Convolvulus arvensis*) and *Colletotrichum capsici* controlled morning glory (*Ipomoea* spp.). The naturally occurring fungus *Phoma macrostoma* has been studied for control of dandelion (*Taraxacum officinale*), Canada thistle (*Cirsium arvense*), chickweed (*Stellaria media*) and scentless chamomile (*Matricaria perforata*), and its effect is equivalent to the industry standard synthetic herbicide pendimethalin [21]. One group of important bioherbicide candidates, soilborne fungi, significantly reduced weed populations by causing seed decay prior to emergence or killing seedlings shortly after emergence [22]. In a study by [23], *Trichoderma virens* (*Gliocladium virens*) colonized composted chicken manure and significantly reduced the emergence and growth of redroot pigweed (*Amaranthus retroflexus*) and broadleaf weeds in fields of horticulture crops.

Bacteria have also been studied in order to cause diseases in weeds, such as *Xanthomonas campestris* that is registered to control annual bluegrass [24]. Pathogenic bacteria *Xanthomonas campestris* pv *poannua* and *P. syringae* pv *tagetis* have been developed as bioherbicides to control annual bluegrass (*Poa annua*) and Asteraceae weeds, respectively [25]. The phytotoxin produced from a crude extract of *Pseudomonas syringae* reduced root and shoot growth of weeds in newly-established "Stevens" cranberry bogs [26]. In greenhouse and field tomato studies, applying the fungus *Myrothecium verrucaria* as a bioherbicide did not affect tomato growth throughout the growing season but killed 90%–95% of purslane species and 85%–95% of spurge species, and the yield was the same as with conventional herbicide application [27]. Spore suspensions of *Microsphaeropsis amaranthi* and *Phomopsis amaranthicola* alone, or a mixture of both organisms, were used as potential bioherbicides and significantly reduced the weed biomass of waterhemp (*Amaranthus rudis*) and pigweed, thereby increasing the yield of pumpkin and soybean [28]. Two fungi isolated from the parasitic weed dodder (*Cuscuta* spp.), *Fusarium tricinctum* and *Alternaria conjuncta/infectoria*, significantly controlled dodder without affecting cranberry growth, and these two fungi have potential to be used as bioherbicides in organic horticulture [29].

2.2. Bioherbicides from Natural Products

The byproducts of natural sources have been developed as potential bioherbicides to control weeds. Dried distillers grains with solubles (DDGS) is a byproduct of ethanol production that is commonly used as cattle feed, and is a potential fertilizer supplement in horticultural production systems due to its high nitrogen content [30]. Applying DDGS on the surface of potting mix at 800–1600 $g{\cdot}m^2$ significantly reduced the number of annual bluegrass seedlings by 40%–57%, and common chickweed (*Stellaria media*) by 33%–58%, respectively [30]. The DDGS applied on the soil surface at 225 $g{\cdot}m^2$ reduced the number of emerging creeping wood sorrel (*Oxalis corniculata*) seedlings by 25% [31]. A byproduct from corn wet-milling showing herbicidal activity is corn gluten meal (CGM), which has the potential to be used as a natural herbicidal product to control many broadleaf and grass species [32]. The CGM suppressed 22 germinating weed species at rates of 300–1000 $g{\cdot}m^2$, and it caused reductions in plant survival, shoot length, and root development of black nightshade (*Solanum nigrum*), common lambsquarters (*Chenopodium album*), creeping bentgrass (*Agrostis palustris*), curly dock (*Rumex crispus*), purslane (*Portulaca oleracea*) and redroot pigweed when applied on the soil surface in a greenhouse [33]. Mustard seed meal (MSM) (*Sinapis alba* "IdaGold", a member of the Brassicaceae) is a byproduct of the commercial mustard oil pressing process [34]. The MSM contains glucosinolates (GLS) that can be enzymatically hydrolyzed to isothiocyanates, thiocyanate (SCN^-), nitriles, and other compounds. These biologically active compounds are toxic to many weed species [35,36]. Applying MSM to the soil surface of containers at 113, 225, and 450 $g{\cdot}m^2$ reduced the number of annual bluegrass seedlings by 60%, 86%, and 98%, respectively [31]. With a MSM application rate of 225 $g{\cdot}m^2$, the number of emerged seedlings and fresh weight of creeping woodsorrel were reduced by 90% and 95%, respectively. Post-emergence application of MSM at these three rates controlled liverwort from 83% to 97% without negative effects on plant growth [31]. However, there is a limitation to MSM use, because its application rate is 10–20-fold higher than typical granular herbicides used in nurseries [31]. Compared to nontreated controls, MSM application decreased emergence rates of kochia (*Bassia scoparia*), common lambsquarters, and barnyardgrass (*Echinochloa* spp.) by 83%, 73%, and 66%, respectively [37].

Bioherbicides from natural sources have shown great potential in organic production systems. Handiseni *et al*. (2012) [38] found that tomato and pepper seedling emergence in *Pythium ultimum*-infested soils have been improved by canola (*B. napus*) and mustard greens (*B. juncea*) seed meals. Brassicaceae seed meals (BSMs) were used to increase soil inorganic nitrogen and the yields of carrot, which had high efficacy in controlling weeds in organic production [39]. In strawberry production, after applying canola-derived BSM and MSM, the weed biomass of shepherd's purse (*Capsella bursa-pastoris*), Italian ryegrass (*Lolium multiflorum*), desert rock purslane (*Calandrinia ciliata*), and annual bluegrass decreased and strawberry fruit yields increased with BSM treatment, which indicated that BSMs may have potential use in organic horticulture systems as combined bioherbicides and green fertilizers [40]. Fennimore *et al*. [41] found that the combination of steam-disinfestation treatment with soil amendments of MSM showed improved strawberry yield as well as weed and pathogen control. In a lettuce field study, the application of meadowfoam (*Limnanthes alba*) seed meal suppressed weeds and increased lettuce yield and leaf nitrogen content [42]. Onions are poor competitors with weeds, which makes weed management in organically-grown onions difficult [34]. In a greenhouse study, MSM significantly decreased redroot pigweed emergence and slightly reduced total yield of onion, indicating MSM has potential to be used as a weed suppressive amendment in an organic onion production system [34]. In organically-grown broccoli and spinach, the application rate of 4.48 t/ha MSM and soybean seed meal significantly increased spinach yield, but broccoli yield was similar in all treatments [43]. However, application rates of 2.5% MSM and mustard greens seed meal significantly reduced heights and biomass in sorghum, and there was a negative effect of MSM on cotton yield [44]. Therefore, it is evident that the type, rate, and timing of seed meal applications should be considered to successfully manage weeds while producing an organic crop. As evidence, the combination of CGM, clove oil, and sweep cultivation had little impact on weed management for organic peanut production [45]. Russo and Webber (2012) [46] also reported that application of CGM and vinegar did not produce peanut pod or oil yields at levels produced with conventional weed control. Therefore, additional alternative weed control techniques and materials should be investigated for organic peanut production, as one example. In container-grown ornamentals, weed emergence was significantly reduced with DDGS application at 800 and 1600 g/m^2 to the soil surface, with no injury on *Rosa hybrid* "Red Sunblaze", *Phlox paniculata* "Franz Schubert", and *Coreopsis auriculata* "Nana", indicating opportunities for use of DDGS for organically-grown ornamentals [30].

2.3. Bioherbicides from Extracts

Extracts from natural sources may also have potential as bioherbicides. Five dipeptides extracted from hydrolyzed CGM inhibited root growth of germinating weeds [47]. Secondary metabolite extracts from the leaves of *Ailanthus altissima* had inhibitory effects on seed germination and plant growth of *Medicago saltiva* [48]. Rice hull extracts demonstrated a significant allelopathic potential. [49] reported that increasing concentrations of warm water hull extracts from selected rice cultivars resulted in inhibition of barnyardgrass germination, seedling growth, and weight. Nieves *et al*. (2011) [50] also reported that methanolic extracts of *Everniastrum sorocheilum*, *Usnea roccellina*, and *Cladonia confusa* inhibited germination and root growth of red clover (*Trifolium pratense*). Phenolics extracted from the lichen *Cladonia verticillaris* caused changes in the ultrastructure of both roots and leaves of lettuce seedlings, suggesting potential as powerful bioherbicides [51]. Black walnut (*Juglans nigra*) has allelopathic effects, and extracts from walnut have been commercially formulated as a bioherbicide [52]. A black walnut extract-based commercial product (NatureCur®, Redox Chemicals, LLC, Burley, ID, USA) completely inhibited growth of horseweed (*Conyza canadensis*) and hairy fleabane (*Conyza bonariensis*) at a concentration of 33.3%, showing potential as a pre- and post-emergent bioherbicide [52].

Herbs are rich in essential oil content, and essential oil extracts with allelopathic effects can be used for weed management [53]. The essential oils from eucalyptus (*Eucalyptus* spp.), Lawson cypress (*Chamaecyparis lawsoniana*), rosemary (*Rosmarinus officinalis*), and white cedar (*Thuja occidentalis*) significantly inhibited the weed species amaranth (*Amaranthus retroflexus*), purslane (*Portulaca oleracea*), and knapweed (*Acroptilon repens*), and may be applied for biological control of weeds as pre-emergent weed seed germination inhibitor [53]. Onen *et al*. (2002) [54] reported that the essential oils extracted from leaves and flowers of five different plant species (*Artemisia vulgaris*, *Mentha spicata* subsp. *spicata*, *Ocimum basilicum*, *Salvia officinalis*, *Thymbra spicata* subsp. *spicata*) were highly phytotoxic to seed germination and seedling growth of eight weed species from different families (*Agrostemma githago*, amaranth, *Cardaria draba*, *Chenopodium album*, *Echinochloa crus-galli*, *Reseda lutea*, *Rumex crispus*, *Trifolium pratense*). Manuka oil, the essential oil distilled from the manuka tree (*Leptospermum scoparium*), exhibited good post-herbicidal activity for control of the emergence of large crabgrass (*Digitaria* spp.) seedlings, which may be used as a potential bridge between traditional and organic agriculture [55]. Volatile oils from leaves of *Eucalyptus citriodora* caused severe damage to the noxious weed *Parthenium hysterophorus* [56]. The essential oil extracts from *Origanum syriacum*, *Micromeria fruticosa*, and *Cymbopogon citratus* had inhibitory effects on seed germination of wheat, *Amaranthus palmeri*, and *Brassica nigra* [57]. Other plants that have essential oils with allelopathic effects include aromatic plants such as *Rosmarinus officinalis*, *Laurus nobilis*, *Xanthoxylum rhesta*, *Cunila spicata*, and *Artemisia* spp. [58–62].

3. Factors Affecting the Efficacy of Bioherbicide

The efficacy of bioherbicides is the main limiting factor for their use, often due to environmental factors. The humidity requirements for establishment and spread of many foliar and stem fungal pathogens for weed control necessitate the development of special formulations to ensure the effectiveness of agents applied in the field [11]. A long dew period is required by some pathogens for infection on the aerial surfaces of target weeds [63]. Some organisms have limited shelf lives, and they are not suited for long-term storage [64]. *Xanthomonas campestris* pv. *Poannua*, a pathogen causing bacterial wilt of annual bluegrass, was not successfully commercialized due to low performance and variability in efficacy under different environmental conditions [65]. Soil moisture can be an important factor affecting pathogens attacking weeds. Application of a jute fabric to cover soil areas treated with a *Sclerotinia minor* granular bioherbicide to reduce water loss significantly enhanced control of dandelion (*Taraxacum* spp.), white clover (*Trifolium repens*), broadleaf plantain (*Plantago major*), buckhorn plantain (*Plantago major*), ground ivy (*Glechoma hederacea*), and prostrate knotweed (*Polygonum aviculare*) [66]. The influence of moisture was reduced by addition of an invert oil emulsion to conidial suspensions of *Colletotrichum truncatum*, which resulted in 100% control of hemp sesbania (*Sesbania exaltata*) in the absence of moisture in the greenhouse, and in 95% control of hemp sesbania in the field [67]. *Phoma macrostoma* has been registered as a bioherbicide to control broadleaved weed species, and its efficacy on dandelion was significantly increased by 10%–20% by amendment with nitrogen fertilizers [68].

The bioherbicide application method should be considered for enhancing efficacy of the biocontrol agent, including attention to spray droplet size, droplet retention and distribution, spray application volume, and the equipment used [69]. The application distribution pattern and pressure are important considerations for determining the quantity of bioherbicide applied [70]. Retention of spray droplets is affected by surface characteristics and morphology of the weed, its biotypes, the adjuvants used in the solutions, travel speed, and droplet size [71]. Smaller droplet sizes of *Colletrotrichum truncatum* resulted in greater efficacy in controlling scentless chamomile (*Matricaria perforata*) [72]. Application of bioherbicides with different nozzles affected the disease incidence and development on waterhemp [73]. Innovations such as dual nozzle sprayers, and the use of compressed air rather than CO_2 to minimize the acidification of the spray solution, may have impacts on bioherbicide efficacy [69].

Other factors, such as the spectrum of the bioherbicide, whether broad or targeted to specific species, the type of formulation, and if it involves amino acid-excreting strains, can significantly affect efficacy. Broad-spectrum bioherbicides may show different efficacies in different regions. That can be altered, as the spectrum of *Alternaria crassa* was broadened by combining it with fruit pectin and plant filtrates [74]. Another method to broaden the spectrum of bioherbicide is to combine multiple pathogens. By combining *Alternaria cassiae*, *Phomospsis amaranthicola* and *Colletotrichum dematium*, weeds such as pigweed (*Amaranth* spp.), sicklepod (*Senna obtusifolia*), and showy crotolaria (*Crotalaria spectablis*) were effectively controlled [75]. Chandramohan and Charudattan (2003) [76] also found that a mixture of three pathogens, *Drechslera gigantia*, *Exserohilum longirostratum*, and *Exserohilum rostratum*, successfully suppressed the growth of seven weeds in citrus groves in Florida. Amendment of bacterial pathogen aqueous suspensions with surfactants has been studied for helping bacteria efficiently invade plant leaves and broaden host range [25]. Types of formulations using emulsions, organosilicone surfactants, and hydrophilic polymers have advantages and disadvantages in enhancing the efficacy of biotic agents and ease of application [69]. Emulsions may improve efficacy and consistency of weed control by predisposing weeds to a bioherbicide agent [69]. Organosilicone surfactants, such as Silwet L-77, facilitate direct entry of bacterial cells and small spores into weed tissues [69]. Hydrophilic polymers, including numerous types of natural and synthetic polymers, have different levels of water-holding qualities. However, formulations composed of expensive materials increase the cost of bioherbicide products. In addition, some materials used in these formulation are toxic to human health [69]. An abundant quantity of amino acids has the potential to terminate plant growth. Therefore, the selection of fungal strains that are able to produce significant quantities of amino acids is becoming a new technique to control weeds [77]. Valine excretion by mutants of *Fusarium oxysporum* controlled *Cannabis sativa* by 70%–90% compared to 25% by a wild type isolate [77].

In addition to bioherbicide efficacy, the high cost and the potential human health threats are some other limitations for use of bioherbicides. Although some pathogens are highly effective in controlling a number of weeds, they may also produce undesirable mammalian and avian toxins [11]. *Myrothecium verrucaria* was effective for weed control as a result of the production of herbicidal metabolites; however, the mammalian-toxic macrocyclic tricothecenes were also simultaneously produced, presenting a severe human health hazard [78]. A fungal pathogen, *Fusarium tumidum*, a potential bioherbicide for gorse (*Ulex europaeus*) and broom (*Cytisus scoparius*), also produced tricothecenes [79]. With the relatively small market at present, and the high cost of maintaining registration, bioherbicides may be dropped from production, like DeVine (*Phytophthora palmivora*) that provided 95%–100% control of strangler vine [80,81]. Although the demand for more environmentally-friendly strategies and bioherbicides for weed control is increasing, there have been few bioherbicides successfully registered and commercialized in North America due to these limitations.

4. Conclusions

Lacking few effective bioherbicides, the integration of biological controls into current weed management systems may be an effective alternative for organic horticultural production. Bioherbicide technology could be used as a component in integrated weed management strategies to help avoid herbicide resistance, reduce production costs, and increase crop yield in organic horticulture. While there have been significant efforts to develop bioherbicides, few have been registered for use. Future research should focus on the development of more cost-effective and efficient bioherbicides, as well as the optimization of their use in production systems.

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