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BIO-HERBICIDES: DEVELOPMENT, USE AND ELUCIDATION OF THE FACTORS AFFECTING THEIR EFFICACY

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ABSTRACT

Weeds belong to diverse groups of exotic and native grass species and are considered problematic for significant crops such as *Cyperus rotundus* or Dhela; *Avena fatua* or Jungli Jai are considered one of the most dangerous weeds of wheat around the world. The agricultural product loss recorded in Pakistan due to weeds are estimated a 17-25% yield loss in wheat due to weeds, 20-45% loss in maize, 13-41% loss in cotton, 20-63% loss in rice, 10-35% highest loss in sugarcane, and 25-55% in pulses. Many weeds inhibit the growth of other plants with their allelopathic effects through the release of specific inhibiting substances in the topsoil. Several weeds provide them as alternate host plants for various types of insects, pathogenic fungi, bacteria, and viruses that may attack the crop. To meet food demand in the increasing population in upcoming years is a big challenge in Pakistan. The first use of biological control of weeds was reported in 1971, the biological product used to control weeds was called bio-herbicide. It is eco-friendly, safe for non-targeted organisms, practically easy to use, and not involved in health-related risks of humans and animals. This review tried to explain the role of bio-herbicides in the control of weeds.

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INTRODUCTION

Many factors reduce agricultural production; some of the key factors are imbalance or untimely application of fertilizers, growth of weeds, insect pests, microorganisms, diseases, and stress-related abiotic factors (Semenov and Halford, 2009). Weeds are defined as any plant growing out of its desired place. Such a diverse group of exotic and native grasses species is considered problematic for major crops (Chandramohan and Charudattan, 2001, 2003). Some weeds are enviable and economically important in particular situations. The agricultural product loss recorded in Pakistan due to weeds is estimated to 17-25% yield loss in wheat due to weeds, 20-45% loss in maize, 13-41% loss in cotton, 20-63% loss in rice, 10-35% highest loss in sugarcane, and 25-55% in pulses. Many weeds inhibit the growth of cultivated plants with their allelopathic effects through the release of specific inhibiting substances in the topsoil. Several varieties of weeds provide them as alternate host plants for various types of insects, pathogenic fungi, bacteria, and viruses that may attack the crop. To meet food demand in the increasing population in upcoming years is a big challenge in Pakistan (Mahajan, 2018). Bio-herbicides' importance has risen even more due to frequently experienced food shortages due to weeds, insect pests, and diseases. The losses in yield depend upon weed species, degree, and the duration of weed infestation in the field. Because of their good adoption and heavy seed-bearing capacity made them problematic and difficult to control. Some weeds such as *Avena fatua* (Jungli Jai), *Cyperus rotundus* (Dhela), Bird's seed grass (Dumbi sitti), goosefoot (Bathu), and field bindweed (Lehli) are known as high damaging weeds of wheat crops (Estrada-Campuzano et al., 2012). The major weed problems in wheat cultivation are grassy weeds, and under good conditions, weeds grow rapidly and densely, which inhibit the growth of wheat plants. Weed problems are often controlled with chemical herbicide sprays and commonly controlled through mechanical or chemical methods. Hand weeding is still practiced in countries where labor costs are relatively cheaper and easily available. However, chemical sprayers are a widely used and most effective method in controlling weeds. The deliberate use of living organisms processes and products to inhibit the growth of other living organisms is termed as biological control. Natural living agents such as fungi, viruses, bacteria, nematodes, and protozoa-based herbicides are termed as bio-herbicides. While if the user agent is a fungus, it is known as mycoherbicide (Aneja et al., 2013). Two primary fields of biological weed control applications are classical and inundative. Classical biological weed control methods refer to natural predators, pest species, or pathogens released with anticipation (TeBeest, 1996). Inundative biocontrol of weeds invokes bacterial suspensions or fungal spores with different applications as propagating materials (Hoagland et al., 2007). Using bio-herbicides with direct application of plant pathogens in annual crops is a major substitute for conventional chemical input strategies (AULD et al., 2003; Caldwell et al., 2012). In massive inundative inoculations with virulent spores, host-specific fungi can effectively manage weeds as bio-herbicides (Templeton and Smith Jr, 1977). The cost of development of bio-herbicides was reported lower than developing a chemical herbicide (Li et al., 2003). *Cyperus rotundus* (Dhela; family Cyperaceae) and *Avena fatua* (Jungli Jai) are the most promising weeds of a wheat crop, causing a decline in wheat production and low product quality in Pakistan. Purple nutsedge (*C. rotundus)* is highly competitive and causes seed cotton yield reduction by 62-85% compared with no purple nutsedge control treatments (Bryson et al., 2003; Guantes and Mercado, 1975; Kondap et al., 1982; Simkins and Doll, 1980; Singh and Singh, 1977) With some exceptions, agronomic crop yield reductions from nutsedge weed were 0-42% marked low to moderate. Yields lost for beans, garlic, okra, and squash vary from 41-89%, exceeding other crops with 0-53% loss (Stilwell and Sweet, 1974; William, 1973). *A. fatua* is among the ten worst annual weeds worldwide (Darbyshire, 2000; Darbyshire, 2003). It is a weed of clay to clay loam soils of temperate agricultural regions (Holm et al., 1991). The ultimate effect of weed competition is stunted growth and yield losses of major crops, i.e., wheat. *A. fatua* is a major weed of wheat in Pakistan and causes huge losses on a national level (Koide and Lu, 1992). *A. fatua* competition reduced cereal crop yield by as much as 70%. In spring wheat, yield losses from *A. fatua* interference ranged from 10-60%, depending on cultivar, plant density, agronomic production factors, and environmental conditions (Carlson and Hill, 1985; Cudney et al., 1991; Kirkland and Hunter, 1991). In Australia, *A. fatua* was twice as competitive with wheat as the most abundant grass weed (Pannell and Gill, 1994). The damage due to *A. fatua* is due to its greater rooting ability than wheat crops (Sorkhy Lalelo et al., 2008). *A. fatua* occurred mainly below ground, particularly soil nitrogen (Klingman et al., 1975; Satorre and Snaydon, 1992). Bathoo (*Chenopodium album* L.) and Jungle palak (*Rumex dentatus* L.) are the weeds that are most commonly available in the fields of wheat in Pakistan (Siddiqui and Bajwa, 2001). In wheat fields, mechanical methods are becoming more expensive and laborious to manage weeds. While, over the last 20 years, chemical control methods have created weeds that are resistant to herbicides chemical (Friesen et al., 2000). Because of these drawbacks, over the past couple of years, interest has increased in integrating biocontrol methods with other chemical strategies (Amsellem et al., 2001). Alternaria genus has more than 60 species, mostly parasites that live on saprophytes and plants.

Alternaria, such as *Alternaria eichhorinae,* causes several weed diseases and was recorded as a pathogenic fungus of common water hyacinth (*Eichhornia crassipes*) in Kenya, Sudan, Egypt, as well as many other states (Shabana, 2002). Fungus *A. cassia* has bioherbicidal ability to manage *Senna obtusifolia* (coffee weed) (Pitelli and Amorim, 2003). Various *Alternaria alternata* isolates have been originated as biocontrol of plants. The fungus *A. alternats* has two isolates found as promising biocontrol agents for *C. album, R. dentatus* and, especially when the suspension is made in canola oil emulsion in Pakistan.

History of bio-herbicides

Three genera of fungi received more concentration as bio-herbicides within the scientific literature. From the genus *Colletotrichum, Colletotrichum truncatum* specie has been investigated to control *Sesbania exaltata* and *C. orbiculare*. *Eichhornia crassipes*, the worst water hyacinth weed that continues to pose problems to the agriculture sector around the world (Den Breeÿen, 1998). Efforts were made continuously to control this weed mechanically and chemically. There was a biocontrol program ongoing in South Africa against the weed. Later, it was recognized that this weed could be controlled with suitable agents. *Acremonium zonatum*, *Cercospora piaropi, A. eichhorniae*, *Neochetina eichhorniae* water hyacinth weevil, and other individual pathogen interactions were tested. An integrated weed management approach through agents of insects and different combinations of pathogens combined with mechanical and chemical control to achieve the precise, practical, and sustainable level of controls appears. For very effective control of water hyacinth, these appeared essential in management. The *Salvinia molesta,* established in the USA, is a serious aquatic free-floating fern weed in South America (Jan, 1999). In 1995, the first plant was reported outside the cultivation in the southeastern South Carolina pond. In South Carolina, the 1.5 acre population was eradicated. Other infestations of plants were reported weekly in the USA. In Houston 1997, plants were observed at a school-yard demonstration pond. Three major river drainages of Louisiana and Texas. Fungus *S. molests* grows so rapidly that its invasiveness becomes an alarming cause, forming very dense mats that cause the choke of waterways and lakes (Jan, 1999). A single plant can produce enough mat covering thicker than 100 square kilometers within three months. The sodium succinate chemical addition against *Sclerotinia minor* fungus growth, for that purpose media, can be used to produce oxalic acid (Briere et al., 2000). The cultures developed on enriched media with sodium succinate can cause larger necrotic tissue growth with dandelion application than those grown on nonsodium succinate enriched media. Host tissues are acidified by oxalic acid, interfere with polyphenol oxidase (PPO), and enable cell wall degradation normally assists in plant resistance systems (Magro et al., 1984). Many methods and media are found for the fungus *Dactylaria Higgins* (Wyss et al., 2001). The Multiple pathogen bio-herbicide approaches were developed, a cocktail of three fungi against many invasive and native grasses. The cocktail of three fungi is *Drechslera gigantean* Heald and Wolf*, Exserohilum rostratum* (Drechsler) Leonard and Suggs and *E. longirostratum* (Subram.) isolated from three different kinds of grasses at different locations in Florida (Chandramohan and Charudattan, 2001). The pathogenic ability of these fungi to a primary host and then several alternative hosts overlapping make this fungi bioherbicidal against several kinds of grasses. It is also claimed that using more than one pathogen in the cocktail and broad-spectrum activity would make the level of weed control faster. The Mycotech, a *Chondrostereum purpureum* strain HQ1 registered with PMRA Registration No. 27019 in 2002, and the EPA with Registration No. 74128 2 in 2005, where PMRA registration ended by 2008. *Puccinia thlaspeos* fungus was registered in 2002 with the product name of Woad Warrior (Thomson and Kropp, 2004). This obligate parasite fungus was used to control *Isatis tinctoria* Dyer's woad. It required a living host to replicate. Inoculum of this fungus can be obtained from ground, dried plants of the targeted weed. It is not available anymore. Evaluation of these fungi over the past five years confirmed that they are only effective under field conditions for controlling weeds and are pathogenic to one or more weeds at different levels under some specific range of host (Chandramohan and Charudattan, 2001, 2003; Chandramohan et al., 2000; Chandramohan et al., 2002a; Chandramohan et al., 2004; Chandramohan et al., 2002b). The *D. gigantea* with *Bipolaris sacchari*, another fungus of weeds, tested and effectively suppressed cogon grass's growth. These fungi could also suppress the growth of weeds when planted in combination with Bahia grass (Yandoc et al., 2004). European Parliament, 2014 illustrated an even significant number of microbes and their derived chemicals that have been registered with the United States Environmental Protection Agency (EPA) for the crop and other ecological management. The 53 products were registered from 1996 to 2010 by the Environmental Protection Agency of the United States. The European Union approved no more microbes to control weeds (Yandoc et al., 2004). Bewick et al. (1989) discovered a fungus named *A. destruens,* a pathogen of dodder, and discovered it as a bio-herbicide. Smolder is the largest bio-herbicide registered in the USA commercially developed and registered by Loveland Products, Inc., Greeley, Colorado, and registered from this fungus. Field trials have shown that *A. destruens* effectively alleviated dodder inflicted damages under field conditions (Cook et al., 2005). The aim was to develop the best management practices by using a single key component called Smolder for dodder that can be killed with *A. destruens* after five weeks of application in the field. The fungus *A. destruens* strain 059 was registered with a product named Smolder WP and Smolder G (Cook et al., 2005). However, originally this product was isolated from *Cuscuta gronovii* that was grown in Wisconsin with unmanaged conditions. This fungus was intended for the control of species of dodder *Cuscuta* spp. Weevils such as Sands, Calder, and *Cyrtobagous salviniae* from southeastern Brazil showed promising results for managing *Salvinia molesta, and Cyrtobagous salviniae* have been released for complete management of *S. molesta* in the tropical areas of India, Australia, Namibia, Botswana, and Papua New Guinea. *C. salviniae* succeeded in managing the potential infestation of *S. molesta* in the USA. The templates herbicides can be well established by a plant hormone, a derivative. Three species of the *Phoma* genus received much consideration as biological weed control agents. A fungus *Phoma herbarum* originally isolated in Southern Ontario from the leaf lesions also used for the dandelions control in turf (Neumann and Boland, 1999; Stewart-Wade and Boland, 2005) *P. macrostoma* fungus possesses similar purposes and particularly inhibits the germination of plants (Bailey et al., 2013). The macrolides and other molecules within the tetrameric acid family used as templates for the synthetic novel herbicide's development had received significant attention (Zhao et al., 2011). The anthraquinone stain separated from the *Phoma macrostoma* strain showed an herbicidal effect on numerous weeds prominent in Central India. The mechanism of phytotoxic effects on these compounds is not fully characterized (Yoshinari et al., 2010). The genomes of *Colletotrichum gloeosporioides* and *C. orbiculare* contain a certain number of applicant genes associated with pathogenesis tests (Gan et al., 2013). The control of broad leafy weeds in Canada and the USA, *Phoma macrostoma* species strain was registered in turf systems (Evans et al., 2013). The bioherbicidal activity of 64 strains of *P. macrostoma* and 94-44B strain was found partial to a group of strains (genetically homogeneous) that were isolated from the thistle of Canada (Pitt et al., 2012). The *P. chenopodicola* genus is potential control for the *Chenopodium album* (Cimmino et al., 2013). Two species within the genus *Sclerotinia* have shown the potential of weeds control. The effective control of dandelions by *Sclerotinia* fungus in greenhouses within or without turf species. Its minor application under open field conditions confirmed the outcomes (ABU-DIEYEH and WATSON, 2007). A strain of *Sclerotinia* under product Sarritor was investigated for the care industry of the Canadian lawn. Though, this product is no more commercially available now (Watson and Bailey, 2013). The *Sclerotinia sclerotiorum* tested relative to *Sclerotinia against Cirsium arvense*, having phytotoxic activity (Skipp et al., 2013). A role of virulence has been performed by *Sclerotinia* minor through oxalic acid production and *Sclerotinia sclerotiorum* on their respective host plant (Magro et al., 1984). In soy and tobacco cell cultures, oxalic acid's low concentration inhibits hydrogen peroxide discharge, a plant defense molecule (Cessna et al., 2000). The bio-herbicides of many fungi registered in forestry and ecosystem management in the USA and Canada (Bailey, 2014). It is the formulation of *Phytophthora palmivora* fungus (Bailey, 2014). *P. palmivora* was isolated originally from *Morrenia odorata*in Florida. The same species were controlled by this fungus in citrus orchards (Ridings, 1986). The wheat straw, bagasse, and rice straw to molasses and chickpea flour for the mass culturing of fungus *Alternaria alternate*. Fungal isolates have bio-herbicides potential against weeds like Jungli Palak (*Rumex dentatus* L.) and bathoo (*Chenopodium album* L.). The *Alternaria alternata* fungus viability test has shown conidia massively produced on preferred suitable substrate against *R. dentates* weed at 4 to 5 leaves stage and *C. album* at 10 to 15 leaf stage revealed a 100% mortality rate at 100% humidity of target weeds (Siddiqui and Bajwa, 2008).

Present scenario regarding development and use of bio-herbicides

The practical application, commercialization, and discovery of particular mycoherbicides were comprehensively developed and evaluated for commercial application purposes (TeBeest, 1996). Early mycoherbicides infected the aerial portions of weeds and their hosts. They consisted of highly virulent plant fungal pathogens resulting in visible disease symptoms externally. Such fungi can also be produced through artificial media methods with mass culture producing inoculums in bulk. It was then used for field applications. Most recent bio-herbicides developed comprising microbes that include obligate fungal parasites, non-phytopathogenic fungi, soil-borne pathogens, pathogenic and nonpathogenic nematodes, and bacteria. Many microorganisms have shown diverse chemical and cultural application requirements compared to early mycoherbicides. Such findings created curious problems even with much potential bio-herbicides availability and targeted weeds in varied habitats. The production, formulation, and application approaches became multifarious and expanded over many years (Charudattan, 2001). Specific standard processing and culturing techniques cannot be implemented to prepare different biocontrol agents. Such techniques were necessary for pathogens to attack targeted weeds that infest the major crops. It is the only unfortunate for developing bio-herbicides, especially mycoherbicides (Charudattan, 2001). The bio-herbicides were extensively implemented to evaluate them for commercial purposes, including regulatory agencies' evaluation and field testing. The original concept of developing bio-herbicides was based on mass artificial culturing of microorganisms to obtain and develop inoculum in bulk amount for inundative application of weeds and on host plants, to achieve a high level of disease rapid outbreak buildup (Charudattan, 2001). Recent bio-herbicide applicants differ from mass production's original characterization and application requirements. For that, the bio-herbicide definition has been redefining Particular living organisms that control selective weeds within a short and specific timeframe in agricultural systems. Various mycoherbicides consist of *Colletotrichum* species fungal pathogens, which can survive saprophytically even in the absence of the host plant. It is significant in artificial culture strategies development for inocula production. Though, some obligatory fungal parasites propagate only on host plants. For example, in the case of *Puccinia canaliculata,* rust fungus is a foliar pathogen of *Cyperus esculentus;* yellow nutsedge is cultured, particularly on weed host plants. The plantation in small plots in field areas or under greenhouses is done from where uredospores vacuum-harvested and stored in bulk earlier to mycoherbicides development (Phatak et al., 1983). The vacuum-harvested uredospores can be implemented through center pivot irrigation systems technology in the field. *Chondrostereum purpureum*, a fungal pathogen, had a myco-herbicide product named 'Eco-Clear' that can be applied to stumps and wounded branches of tree species of weeds that increase in woody tissues decay by inhibiting the resprouting process. The management of multiple weeds complex in row crops. It deals with transgenic crops using resistant herbicides that eventually develop such weed biotypes resistant to herbicides after the repeated appliance of the same herbicide in a particular central crop field area. *Lolium rigidum* is rigid ryegrass that developed glyphosate resistance after multiple applications of glyphosate to control grass weeds (Powles et al., 1998).

An example case is *Cyperus rotundus*

Cyperus rotundus is a perennial, persistent weed plant of subtropical, tropical, and temperate regions worldwide. These weed plants have slender leaves that reach 20-60 cm in height. Leaves are shorter and connected with modified, underground modified stem rhizome. The *Cyperus rotundus* weed produced underground basal bulbs, tubers, and rubbery roots. Rhizomes were fleshy white with brittle leaves that became fibrous and woody. They were darker brown with long creep stolen numerous (Sivapalan and Jeyadevan, 2012). It has a terminal inflorescence with numerous flowers, which produced only a small number of seeds. Therefore, seeds were not considered the main foundation of proliferation in *C. rotundus.* The flowering period of *C. rotundus* is between April-October (Flora of Pakistan, 2014). The modified, underground parts such as rhizome, corms, and tubers were the main propagating parts of *C. rotundus* (Brosnan and DeFrank, 2008; Lati et al., 2011).

Factors affecting efficacy bio-herbicide

The study from the screening stage to field conditions faced numerous unique challenges. The most common challenge reported was the availability of continuous moisture during the infection of biocontrol candidates (Boyette and Hoagland, 2015).

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Moisture

A study on bio-herbicides technology reports that more than twelve hours of dew period is required for bio-herbicides to infect the respective host successfully. A prolonged leaf wetness period was necessary for the successful infection of *Phoma herbarum* dandelion. Aqueous inoculants were integrated with vegetable oil emulsions. On the other hand, such additives were found phytotoxic. They obscured the benefit of infection that their addiction may cause. Dawn or dusk application with timely inoculants to prolong the leaf wetness period was recommended as a straightforward approach to maximizing contagion under environmental fluctuations. To prolonged-field survival of biological weed control organism's introduction, a granular application technique may have an advantage by moisture and nutrients provision with a more gradual infection rate (AULD et al., 2003).

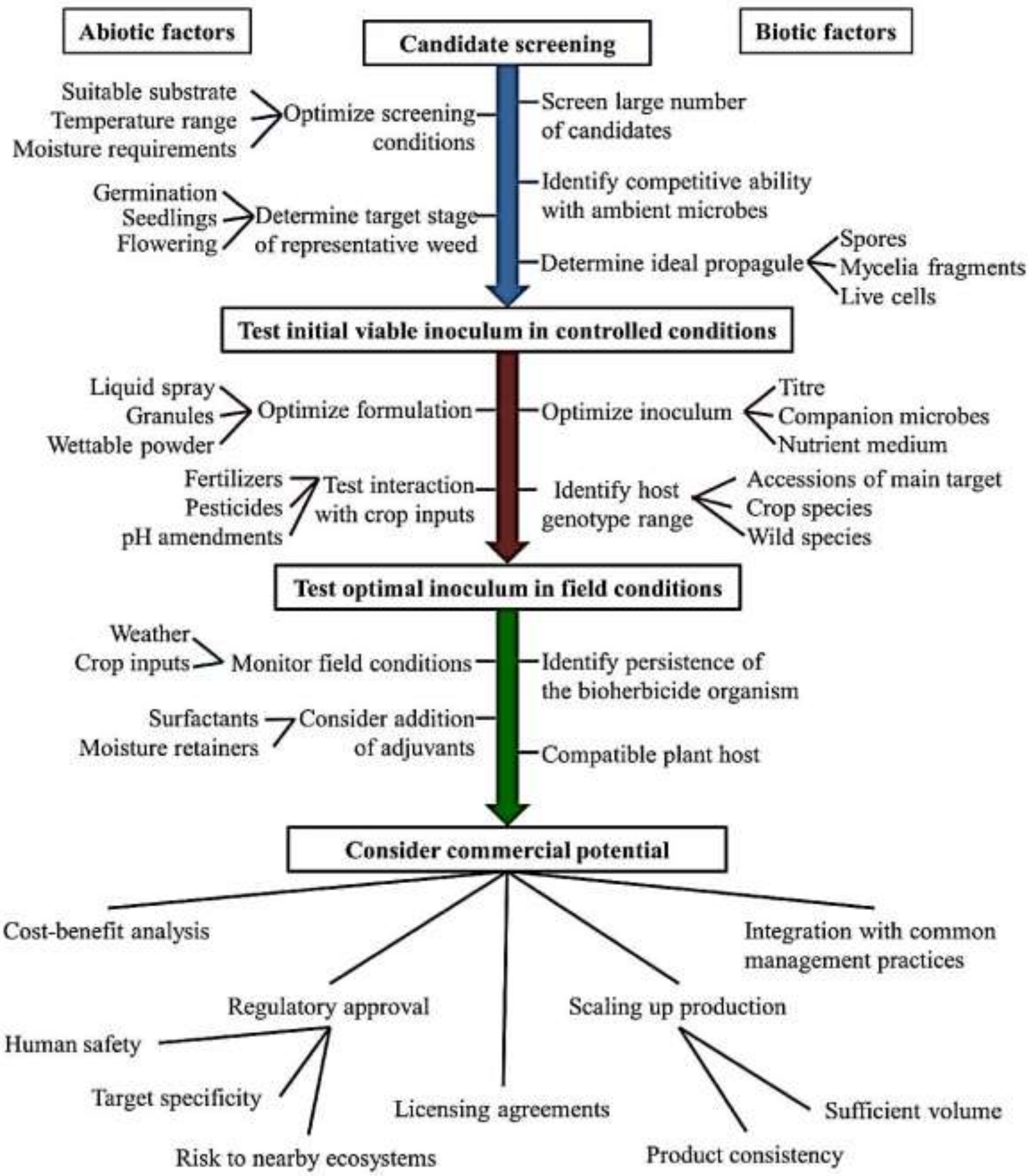

Figure 1. Flowchart of Bioherbicides.

Temperature

The relationship between humidity and temperature has significant consequences on the success or the failure of pathogens infection (Ghosheh, 2005). They may alter their efficacy level to some extent. Cold air retained less total moisture level. Elevated humidity at a lower temperature is usually more favorable for the successful colonization of bio-herbicides. It also decreases the evaporation rate and increases the period of leaf wetness for inoculant application (Casella et al., 2010). The efficiency of a bio-herbicide product named Sarritor was highest below 20°C temperature and at a high relative humidity rate (Siva, 2014). Inoculants testing media with a range of population density through the exploration of biocontrol methods is not a common practice these days (Romero et al., 2001). Bio-herbicides' interaction with pesticides and fertilizers could affect the infectiousness of biocontrol applicants (Boyetchko, 1997).

Conclusion and future aspects

Weeds are a significant threat to the natural and agricultural environment. Because of their diverse habits of intrusiveness with crop growth, weeds reduced crop productivity. Both weeds and crops compete for limited growth factors such as space, solar energy, nutrients, and water. This competition obstructs the crop agronomy processes in the field. Weeds are resilient and dynamic in growing environments. They hastily grow, then the crops caused productivity losses. The yield losses depended on weed species and weed infestation in the field. *Avena fatua* (Jungli Jai) and *Cyperus rotundus* (Dhela) are highly damaging weeds due to their excellent adoption and heavy seed-bearing capacity to the wheat crop. Biological control programs aim to control these weeds only in the agricultural fields reasonably fail because surrounding wild populations caused weeds to re-infest agriculture fields. So, it would be frugal to combine classical, inundative biological control methods depending on the situation. A biological control refers to introducing such selective organisms into an ecosystem to manage unwanted numeral species. *C. rotundus* and *A. fatua* are noxious weeds of tropical and subtropical regions. *C. rotundus* weed is dispersed around tropical and sub-tropical regions of the world. This weed interferes in 52 crops of 92 countries. Both weeds are considered the world's worst, with extensive branches of wheat in Pakistan and worldwide. It was perennial sedge having strong apical dominance. It increased rapidly using a vast underground network of tubers which multiplies rapidly through an extensive underground tuber. Bio-herbicides are phytopathogenic microorganisms or microbial phytotoxins, useful for biological weeds management. Bio-herbicides have defined the use of naturally occurring plant pathogens that cannot be genetically modified and are suitable in organic agriculture. The relationships between agriculture, soil, and pathogens are better implicated than agriculture and climate. It may not be possible to change the climate. Still, it is possible to adopt particular agriculture approaches that aim. These fungi are also a source of many worst diseases infestation in vegetables and major growing crops. Bio-herbicides control of *C. rotundus* and *A. fatua* weeds should not be rejected. Eradication of *C. rotundus* and *A. fatua* is highly unlikely manually due to the plant's extensive distribution resilience, less registered herbicides, and the indisposition of grazers to obliterate important plant pastures. Another major problem is rapid reinfestation by *C. rotundus* and *A. fatua* after herbicide treatments in wheat crop. The high cost of containments, herbicides application, and no alternative approaches to particular weed controls were significant problems in the field.

AUTHORS' CONTRIBUTIONS

All the authors contributed equally in collecting the information and compiling the review article.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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