BIO-HERBICIDES FOR SUSTAINABLE AND ECO-FRIENDLY WEED CONTROL: A REVIEW.

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Abstract

Weeds are the major cause of crop yield reduction. Synthetic chemical herbicides are very effective in weed control and accomplish the weed reduction up to large extent but, due to resistance development, they become less effective for weed control. There is a need requirement for suitable alternative of chemical herbicides. Bio-herbicides are upcoming products for sustainable weed control. Bioherbicides are the new approach, originated from living organisms or natural metabolites of plant for weed control. Bio-herbicides includes microbes- fungal, bacterial and viral pathogens and plant based products includes plant extract and essential oils. The bio-herbicide inhibit of physiological activities like nutrient uptake, photosynthesis etc and disrupt cellular functions like cell wall and cell membrane, hormone and toxic production etc. Different types of formulation have been developed to enhance the shelf life of different bioherbicides for successful commercialization.

Introduction:

With the increase in population agricultural land decreases which may cause food shortage in upcoming years. There is an urgent requirement for high agricultural yield by improved and safe practices. There are many agrochemicals are available against different types of agricultural pest. These agrochemicals are competent to control the various crop pests like insects, fungi, pathogens, weeds etc. Among these pest, weed problem is the major problem and results into 34% loss in crop yield.

Weeds are the unwanted plant population and compete to the main plant for the resources and major cause of low crop yield. After world war II selective herbicides, MCPA and 2,4-D, introduction considerably reduced the weed losses without harming the main crop (Mithila et al., 2011). At present, 25 herbicide sites have been discovered but still weed resistance problem have been reported despite of selectivity (Heap, 2015). Transgenic crop was adopted worldwide to combat with this resistance problem and replace the former chemical control methods (Green and Owen, 2011; Beckie, 2011). Continuous development of advance control methods are required for for overcoming the problems related to weed control and maintenance of agricultural yields.

Weed control involves integrated weed management practices by which weed losses can effectively reduced. There are various methods of Weed management which involves direct and indirect methods (Chikowo et al. 2009; Marshall et al., 2003;). Indirect methods weaken the weed by reducing their
vegetative and reproductive growth. Direct methods involves weed control by destroying the weed plant by manual, mechanical, biological and chemical methods (Chauvel et al., 2012). These existing weed control methods have some limitations and not competent towards the sustainability of modern agricultural practices. Therefore, new weed control methods are being explored which are environment friendly as well as efficiently control the weed problems.

**Bioherbicides: safe approach:**

Bio-herbicides are defined as the substance which reduce the weed population by bio-organisms like microbes, pathogens or natural metabolites. It consists of plant based natural products, pathogens, and other microbes used for biological weed control. Bio-herbicides are the new strategy to minimize the shortcomings of the existing conventional herbicides as shown in fig.1.

This approach is based on classical and inundative strategy. In classical strategy microorganisms establish, multiply and spread and persist in the ecosystem for the infestation against target weed (Shaw et al., 2009; Dane and Shaw, 1996; TeBeest, 1996). The main objective of this strategy is to maintain the weed population below the threshold level rather than completely eradication. On the other hand, in inundative control strategy fungal spores or bacterial suspension is applied for destroying weed population this application is not persistent (Auldetal., 2003; Caldwelletal., 2012).

**Classification of Bioherbicides:**

Bioherbicides may be classified on the basis of their source as given below:

**Bioherbicides**

- **Pathogens**
  - Fungal
  - Viral
  - Bacterial

- **By product of plants**
  - Allelochemicals
  - Plant extracts
  - Essential oil

**Fig.1:** bioherbicides – safe approach

**Fig.2:** classification of bio-herbicides
Pathogens as bioherbicides:-
There are approximately 200 different plant pathogens have been identified by plant pathologists and weed scientists (Arton J. et. al. 1999). Pathogens used as herbicides also known as Mycoherbicides. Mycoherbicides are host specific, non resistant and will reside in soil for longer duration of time. There are 37 mycoherbicides and 8 application techniques of these mycoherbicides are currently available.

Fungal Pathogens as bioherbicides:-
Fungi are the most common plant pathogens for weed control. Fungal pathogens are the most promising alternative of synthetic chemical herbicides for weed management systems (Evidente & Motta, 2001; Evidente & Abouzeid 2006). Fungal pathogens weed control is based on their phytotoxic metabolites. These metabolites inhibit the plant pathways and toxic to weed plant cells. Alternaria, Ascochyta, Drechslera, Phoma, Phyllostictica, Pyrenophora, Septoria, Stagonospora, are the most common fungal pathogens for the biocontrol of weeds like Chenopodium album L., Cirsiumarvense L., grass weeds etc. First fungal herbicides are legally market in Canada in 1973.

A fungal pathogen, Phomopsisamaranthicola, potentially used against different species of Amaranthus (Rosskopf et al., 2005). This fungus causes stem and root death of the target weeds. Dactylariahigginsii is a fungal pathogen isolated from Gainesville and effective against purple nutsedge (Cyperus rotundus). This pathogen causes foliar blight, leaf spots and premature shedding of leaves results into reduction of nutsedge growth (Kadir and Charudattan, 1999). The Phoma genus species is another potential fungal pathogen for weed control. P. herbarum, isolated from dandelion leaf lesions for dandelions control (Stewart-Wade and Boland, 2005; Neumann and Boland, 1999;). Dicot plant inhibited by P. macrostoma (Smith et al., 2015). Phomachenopodicola, is the another species for the control of Chenopodium album (Cimmino et al., 2013). Two species of Sclerotinia genus are S. minor, S. sclerotiorum have been identified for weed control (Abu-Dieyeh and Watson, 2007). Sclerotinia minor is the most effective bioherbicide against dandelions with turf species in green house conditions. (Watson and Bailey, 2013). Both S. minor and S. sclerotiorum are phytotoxic agents for creeping thistle (Cirsium arvense) (Skipp et al., 2013). Main phytotoxicant by these two fungal pathogens is the Oxalic acid. Oxalic acid is the inhibitor of polyphenol oxidase (PPO) inhibitor of plant defensinmolecules (Magro et al., 1984).

In addition to these fungulgenuses, Chondrostereum purpureum strains have registered by the US and Canada for inhibiting growth of deciduous tree species in coniferous plantations (Bailey, 2014). Puccinia thlaspeos, is another fungus which was registered with the Environmental Protection Agency (EPA) in 2002 of trade name Woad Warrior specially for the control of Dyer’s woad (Isatis tinctoria). (Thomson and Kropp, 2004). Alternariaestruens strain 059 was registered under the product names Smolder WP and Smolder G with EPA for control of Cuscuta spp. (Cook et al., 2009). Phytophthora palmivora was registered as DeVine, a formulation with the EPA to control Morrenia odorata species in citrus orchards (Ridings, 1986 and Kenney, 1986).

The literature reference of fungal pathogens are given below:-
Table 1:-Fungal pathogens and targeted weed

| Bioherbicide agent | Target weed control | References |
|--------------------|---------------------|------------|
| Phomopsisamaranthicola | Amaranthusspecies | Rosskopf et al., 2005 |
| Dactylariahigginsii | Cyperusrotundus | Kadir and Charudattan, 1999 |
| Phoma genus species | P. herbarum | Stewart-Wade and Boland, 2005; Neumann and Boland, 1999 |
| Phomachenopodicola | Chenopodium album | Cimmino et al. 2013 |
|                  | Cirsiumarvense     | |
|                  | Setariaviridis     | |
| Phomamacrostoma | Dicot plants | Bailey et al. 2011 |
**Viruses as Bioherbicides:-**

Viruses can also be used as bioherbicides in controlling some weeds but due to some constrains they are not effective as fungal pathogens. Viruses have lots of genetic variability and are not target specific (Kazinczi et al., 2006). Tobacco mosaic tobamovirus (TMV), a most popular virus, this virus has potential to kill the tropical soda apple (*Solanumviarum*) (Diaz et al., 2014 and Ferrell et al., 2008). The viral pathogens for weed control are depicted in table no. 2.

**Table 2:-** viral pathogens and targeted weed

| S.N | Viral pathogen                              | Weed control         | References                  |
|-----|--------------------------------------------|----------------------|-----------------------------|
| 1.  | *Araujia* Mosaic Virus                      | Moth Plant (*AraujiaHortorum*) | Elliott et al., 2009       |
| 2.  | Tobacco Rattle Virus                        | *Impatiens glandulifera*     | Kollmann et al., 2007      |
| 3.  | Tobacco mosaic virus                         | *Solanumviarum*         | Farrel et al., 2008        |
| 4.  | Obuda Pepper Virus (ObPV) and Pepino Mosaic Virus (PepMV) | *Solanumviarum*         | Kazinczi et al., 2006      |
Bacteria as bioherbicides:
Many bacterias have been proved as potential biocontrol agent for weeds due to some characteristic features like growth cultures can maintained in liquid, prepared as dry formulation and can genitically modified to improve the bioefficacy. characteristic features shown in fig.3

![Grow in liquid cultures](image1)
![formulated as dried formulation](image2)

**Fig.3:** Characteristics of bacteria as potential bioherbicides

Genetically manipulated:
Many previous investigation have been proved that *Pseudomonas fluorescens* and *Xanthomonascampestris* are most popular bacterial species for weed control. Rhizobacteria *Pseudomonas fluorescens* has ability to suppress weed germination of weed plant which include 8 dicot and 21 monocot species (Banowetz et. al., 2008). *Xanthomonascampestris* is the other bacterial species as weed control agent for annual bluegrass (*Poaannua*) of trade name Camperico (Tateno, 2000 and Imaizumi et al., 1997).

**Table 3:** Different weed controlled by bacterial pathogens

| Bacterial bioherbicide | Weed control   | References                        |
|------------------------|----------------|------------------------------------|
| *Pseudomonas fluorescens* strain BRG100 | *Setariaviridis* | Quail et al., 2002 |
| *Pseudomonas fluorescens* strain D7 | *Bromustectorum* | Kennedy et al. 1991 |
| *Xantonomascompestris* | *Poaannua*     | Tateno, 2000, Imaizumi et al., 1997 |
| *Streptomyces hygroscopicus* | General vegetation | Rupp et al. 1977 |
| *Ralstoniasolanacearum* | *Solanumnigrum* | James T. De Valerio et al., 2011 |
The Mode of action of microbial bioherbicides is represented in fig 4:-

![Diagram showing the mode of action of bioherbicides](image)

**Plants as bioherbicides:**
Many plants based products are also utilized as potential natural agent for weed control. Plants have secondary metabolites or other photochemical which shows inhibit seed germination and other growth processes.

![Classification of plant based products as weed controlling agent](image)

The Plant products can use as weed controlling agent in three form i.e plant extract, essential oil and allelochemicals. These three plant products have been using as potential bioherbicides from last so many decades. This primary mode of action of plant based products is the inhibition of germination of weeds and reduction of plant growth. Plant extract from any part of the plant have many constituents like extracted peptides, secondary metabolites - alkaloids, terpenoids, tetraterpenoids etc.
### Table 4: Plant as bio-herbicides

| Plants | Plant based bioherbicides | Mode of action | References |
|--------|---------------------------|----------------|------------|
| Aglaia odorata | Leaf extract | Inhibit growth of weed plant | Kato-Noguchi et al., 2016 |
| Usnea roccellina, Everniastrumsorocheilum, and Cladoniaconfusa. | methanolic extracts | inhibited root growth and germination of red clover (Trifolium pratense) | Nieves et al. (2011) |
| Rice hull | Hull extracts | inhibition of germination, seedling growth, and weight in barnyardgrass | Ahn et al. 2000 |
| Ammivisnaga (L.) Lam. Khellin and Visnagin | Plant extract | Inhibit seed germination, photosynthesis, cellular activities. | Travaini et al. 2016 |
| lichen Cladoniaverticillaris | Phenolics extracts | change the cellular structure of leaves and roots of lettuce seedlings | Tigre et al. 2014 |
| Black walnut (Juglans nigra) from walnut | Plant extracts | Inhibit growth of horseweed (Conyzacanadensis) and hairy fleabane (Conyzabonariensis) act as a pre- and post-emergent bio-herbicide | Shrestha et al. 2009 |
| Sonchusoleraceus L. | Plant leaves powder | Inhibit seed germination, seedling growth | Hassan et al. 2014 |
| Partheniumhysterophorus | Crude plant leaves extracts | Inhibit seed germination, growth and vigour of whole plant | Pati and Chowdhury 2015 |
| **Essential oils** | | | |
| Artemisia absinthium L | Essential oil | Inhibit seed germination and seedling growth | Fouad et al. (2015) |
| Brassica napus | Se-seed meals | Inhibit seed germination and seedling emergence | Banuelos, 2010 |
| Cymbopogoncitrus | Essential oil | Inhibit seed germination and growth | Fouad et al. 2015 |
| Eucalyptus citriodoraHook | Essential Oil | Inhibit plant growth by stopping the process of respiration, lessen the membrane integrity, premature death of plant due to chlorosis and necrosis. | Batish et al. 2007 |
| Limnanthes alba | Activated seed meal (Isothiocyanate) | Inhibit seed germination | Intanon et al. (2014) |
| Syzygiumaromaticum | Essential oil | Inhibit seed germination, seedling growth, chlorophyll, respiration | Ahuja et al. 2015 |
| Eucalypt (Eucalyptus nicholii), Rosemary (Rosmarinusofofficinalis L.), Lawson cypress (Chamaecyparislowsoniana) and White cedar (Thujaoccidentalis) plants | Essential oil | Amaranth, Purslane and Knapwee germination inhibitors species | SadrollahRamezani et al. 2008 |
Ocimum basilicum, Mentha spicata, Artemisia vulgaris, Salvia officinalis, Thymbra spicata subsp. spicata) Essential oil Inhibit seed germination and seedling growth of eight weed species belongs to different families (Chenopodium album, Agrostemma githago, Cardariadraba, amaranth, Reseda lutea, Echinochloa crus-galli, Rumex crispus, Trifolium pratense).

Leptospermum scoparium. Essential oil Inhibit seedlings growth Dayan et al. 2011

Onen et al. (2002)

Fig. 6: - Mode of action of plant products as weed control agents

Table 5: - Registered pathogens for weed control

| Registered bioherbicides | Pathogen name                  | Targeted Weed                     |
|--------------------------|--------------------------------|-----------------------------------|
| Devine ™                 | Phytophthora palmivora          | Morrenia odorata                  |
| Collego TM(1982)         | Colletotrichum gloeosporiodes   | Aeschynomene virginica            |
| Biomal K                  | Collectotrichum gloeosporiodes  | Malva pusilla                     |
| Wood Warrior K           | Puccinia thlaspeos              | Isatis tinctoria                  |
| Mycotech ™               | Chondrosterem purpureum         | Prunus Serotina Populous euramericana |
| Smoulder K               | Sclerotinia minor               | Dicot weeds                       |
## Comperico

**Xanthomonas Compesteris**

**Poaannoa L.**

In turf

## Organo – sol

**Lactobacillus casei**

**Rhaminouslactis sp.**

**Trifolium repens**

**Trifolium pretense.**

**Lotus corniculah L.**

**Medicagolupulina**

**Oralisacetolla L.**

## Pselouka

**Rape seed oil nonanoic acid**

**Pelargonic acid**

Weeds of potatoes, grapewmes

## Chontrol

**Chondrosterium**

**Perpureum**

Alders and other hardwoods in forest

## Dr. biosedge

**Puccinia cananiculata**

**Cyperus esculantus**

## Slumpout

**Cylindrobasidium leaf**

**Acacia Sp.**

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### Steps involved in registration of pathogenic bio-herbicides:

1. **Step 1:** Field survey for pathogens
2. **Step 2:** Isolation of pathogens
3. **Step 3:** Mass production of inoculums
4. **Step 4:** Determination of disease cycle
5. **Step 5:** Formulation development
6. **Step 6:** Trials in greenhouse for toxicity
7. **Step 7:** Field trials
8. **Step 8:** Registration

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Bioherbicide registration is the complex as compare to synthetic herbicides. Screening of microbial pathogens and phytotoxins is also vary from chemical herbicides. Potential microbial screening is followed by phytotoxicity.
studies which involve greenhouse testing and field application and nontoxic to nontarget plants. Research efforts simplified the process by synthesizing microbial phytotoxic compounds in specific enzyme assay (Boeger and Sandmann 1989). This advance approach resulting some improved products that save the time, cost and labour.

**Constraints of Pathogens as Bio-herbicide:**
There are many limitations of using pathogens as bio-herbicide such as environmental, biological, economic or commercial constraints and technological.

| Limitations                  | Possible Solutions                                                                 |
|-----------------------------|-----------------------------------------------------------------------------------|
| **Biological limitations**  |                                                                                   |
| Most desirable factor for weed controlling agent is the host specificity. It has been discovered that many pathogens are capable to control only one weed species. | I. Application of several pathogens in combined form (Auld et al. 1994)            |
|                             |                                                                                   |
|                             | II. Addition pathogen inoculum with plant extract (Boyette and Abbas 1994)         |
|                             | III. Combining pathogens with synthetic herbicides (Smith 1991)                    |
|                             | IV. Using insects for delivery of weed pathogens to hosts (Charudattan 1986; de Nooij 1988; Kremer 1995) |
| **Environmental limitations** |                                                                                   |
| The most important environmental factors of foliarly applied bioherbicides are:- | These environment factors are very crucial for pathogens in controlling weeds. These factors plays importance role in pathogenic steps as for example moisture is the important requirement for spore germination, mycelia growth so, timing of bioherbicide application coincide with the required factors for retention of water for weed host pathogenicity (Makowski and Mortensen 1990) |
|                             | • Moisture (dew) (Makowski 1993)                                                  |
|                             | • Bioherbicide application time                                                   |
|                             | • Formulations                                                                    |
|                             | • UV radiations, Humidity, and rain water (Leathers et al. 1993).                 |
|                             | • compatibility with synthetic agrochemicals                                       |
| **Technological limitations** |                                                                                   |
| Major obstacle in mass production of microbes are their stability, viability and shelf life of the inoculums (Jackson et al. 1996b). | Formulation give the successful techniques for the innovative mode for easy application and enhancement of the stability of microbes for weed control (Boyette et al. 1996). Formulation contain surfactant and other adjuvants for uniform distribution and adhesion on leaf surfaces (Egley and Boyette 1995). |
| **Commercial/economic limitations** |                                                                                   |
| Microbial herbicides are not economically sound because of deficiency of promising techniques of mass production and synthesis for microbial | Encouragement of bioherbicide research and development work |
Formulations of Bio-Herbicide:-
Bioherbicide formulation is the blending of active ingredient and inert materials for effective delivery of herbicide dose to the targeted host weed plant (Rhodes, 1993). Formulated products of bioherbicides are very useful to improve application, survivability, efficacy and maintaining viability during storage and reduce moisture requirement for germination (Green et al., 1998).

Types of Bioherbicide formulations

Bioherbicides Formulations:-
Two approaches of bio-herbicide formulation are liquid formulation and solid formulation (fig.8).

Liquid formulation:-
These are sprayable formulations these formulations include suspension emulsions, emulsions and polymer based products (Womack et al., 1996). In these formulations water is the transporting medium and adjuvants used assist the active ingredient transport in weed plants (Foy, 1989).

Solid formulation:-
These formulation are applied in soil basically in simple formulations like granular and encapsulated forms (Boyette et al., 1991). Inert material includes grains, clay alginate, charcoal, polymers etc. These are most suitably used as pre-emergence application and can give longer period of activity as controlled release formulation. Solid formulations have higher shelf life than liquid formulations. Granular formulations can give 75% weed control.

Different types of formulation as bio-hebicides are given in (table.7)

Table.7:-Formulations of bio-herbicides

| Bio-herbicides    | Formulations                      | References               |
|-------------------|-----------------------------------|--------------------------|
| Fusarium oxysporum| Encapsulated granular formulation | Elzein et al.;2004       |
| Fusarium spp.     | Encapsulated granular             | Amsellem et al.;1999     |
| Fusarium oxysporum| Gum arabic seed coating           | Elzein et al.;2006       |
| Myrothecium verrucaria | Inverted emulsion formulation   | Yang and Jong ;1995      |
| Colletotrichum orbiculare | Microemulsion                | Klein et al.;1995        |
| Collego and BioMal| Wettable powder                  | Boyette et al.;1996      |
| Alternieramacrospora| Granular formulation             | Walker ;1981             |
| Phomopsis sp.     | Encapsulation of hyphal fragments | Chittick et al.;2003     |
| Alternaria Eichhorniae | Aqueous Mycelial Inoculum     | Shaban et al. ;1997      |
| Colletotrichum orbiculare| water suspension               | Chittick and Auld ;2001  |
| Exserohilum monoceras | Emulsion                      | Zhang and Watson ;1997   |
Future prospects of bioherbicides:-
More work has to be done to improve the most negative aspect of microbial pathogens as bioherbicide is host specificity problem because of narrow host range.

Generation of advance information on allelopathic mechanisms like weed defense and phototoxin production by pathogens.

More attempts are required for covering wide host range via new generation formulations, synergistic combinations and other biotechnological approaches.

Elimination of toxicity problems caused by pathogens like allergic reactions in humans and other animals.

Conclusion:-
Weed pest is the major factor in crop yield reduction and become the main obstacle in meeting the food requirement of increasing world population. Despite of safe and sustainable approach of bio-herbicides, these are not being utilized in agricultural practices. More research and development is required to enhance the activity of bio-herbicides and make it commercialized. Host specificity is the major problem in bio-herbicides because they are not covered the wider range of weed species in field. Bioherbicides should be used as integrated approach to avoid major problem like resistance as well as host specificity. Bio-herbicides may be the main weed controlling agent for promotion of organic farming. So, different new generation formulation techniques can make the bio-herbicide more efficient. Formulation should be developed in combination of different biocontrol agents to cover the wide range of weed species.