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RESEARCH ARTICLE

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). Ttransgenic crop was adopted worldwide to combat with this resistance problem and replace the former chemical control methods (Green and Owen, 2011Beckie, 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

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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.

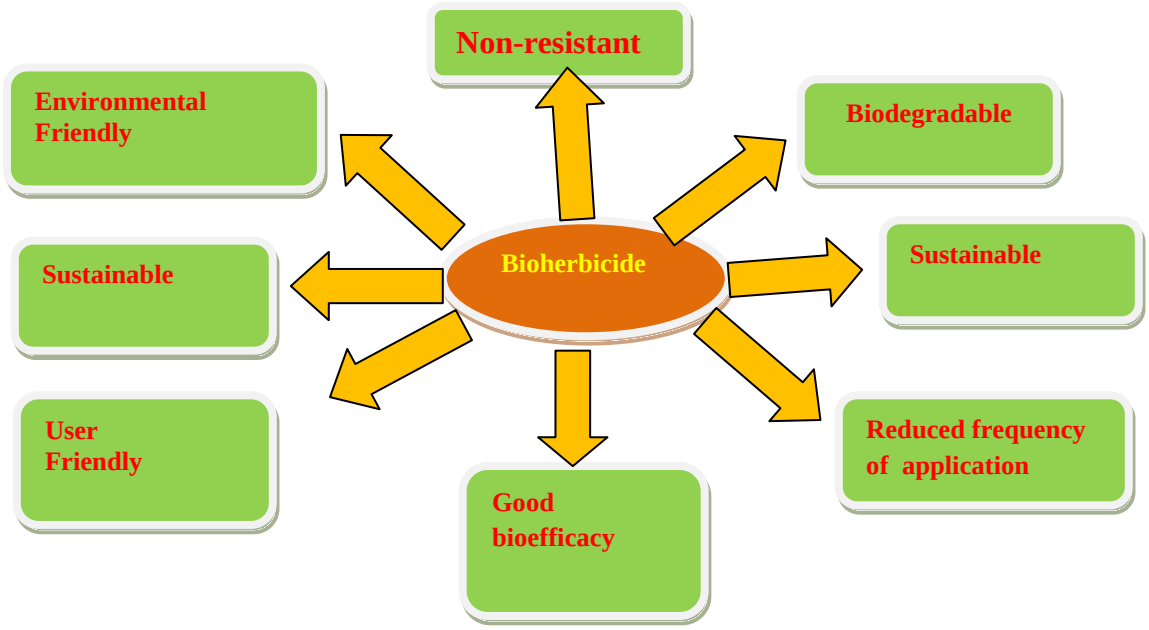


Fig.1:-bioherbicides –safe approach

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:-

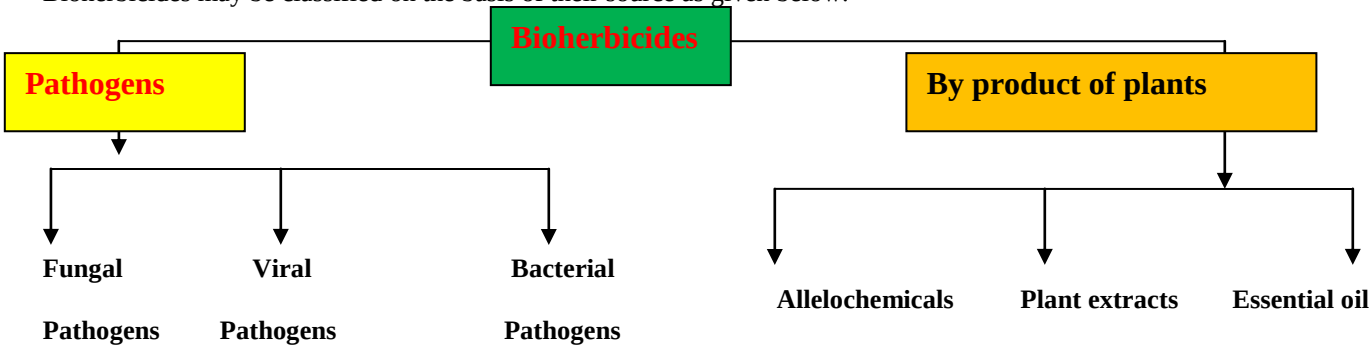


Fig.2:-classification of bio-herbicides

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 fingal 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*, potencially 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 nut-sedge (*Cyperusrotundus*). This pathogen causes foliar blight, leaf spots andpremature shedding of leaves results into reduction of nutsedge growth (Kadir andCharudattan, 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 (**Cirsiumarvense**) (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, **Chondrostereumpurpureum** strains have registered by the US and Canada for inhibiting growth of deciduous tree species in coniferous plantations (Bailey, 2014). **Pucciniathlaspeos**, 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 (**Isatistinctoria**).(Thomson and Kropp, 2004). **Alternariadestruens** strain 059 was registered under the product names Smolder WP and Smolder G with EPA for control of **Cuscuta** spp. (Cook et al., 2009).**Phytophthorapalmivora** was registered as DeVine, a formulation with the EPAto control **Morreniaodorata** 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 Fungal pathogen	**Target weed control**	**References**
Phomopsisamaranthicola	*Amaranthus*species	Rosskopf et al., 2005
Dactylariahigginsii	*Cyperusrotundus*	Kadir andCharudattan, 1999
Phoma genus species	**P. herbarum**	Stewart-Wade and Boland, 2005,Neumann and Boland, 1999;
Phomachenopodicola	*Chenopodium album* *Cirsiumarvense* *Setariaviridis*	Cimmino et al. 2013
Phomamacrostoma	Dicot plants	Bailey et al. 2011

Phomaexigua	*Gautheriashallon*	Zhao& Sharma,2006
Phytophthorapalmivora	**Morreniaodorata**	Bailey, 2014
Chondrostereumpurpureum	Inhibit growth of deciduous shrubs and	Setliff ,2002
Uromycesscutellatus	*Euphorbia esula/virgata*	Caesar,2006
Uromycespencanus	*Naselaneesiana*	Anderson et.al.,2010
Plectosporiumtabacinum	*Gallium spurium*	Zhang et.al.,2002
Fusariumculmonum	*Hydrillaverticillata*	Shabnam et.al.,2003
Fusariumsolani	*Orobancheaegyptica*	Sharma et.al., 2011
Pucciniathalaspeous	*Isatistinctora*	Kropp et.al.2002
Colletotricumtruncatam	*Matricariaperforata*	Graham et.al.,2006
Phompsis Amaranthicola	*Amaranthushybridus*	Chandra mohancharudallan
Neonectria neomacrospora	*Arceuthobium tsugeme*	Rietman et.al.,2005
Myrotheciumverrucaria	*Bramichiaovata*	Boyethe et.al.,2006
Fusariumorysporum	*Cannabis sativa*	Tiourebaev et.al.,2001
Pucciniacorduorm	*cardrumpynocephalus*	Mejri et.al.,2010
Ascochytacaulina	*Chenopodium album*	Ghorbani et.al,2002
Sclerotina Sclerotium	*cirsiumarvense*	Bourdot et. al,2006
Plectosporium alismatis	*Demosonium minus*	Johromi,2007
Alternariaeichhoriniae	*Eichhorniacrassipies*	Shabana and mohammed;2005

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.	Óbuda Pepper Virus (ObPV) and Pepino Mosaic Virus (PepMV)	*Solanumnigrum*	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

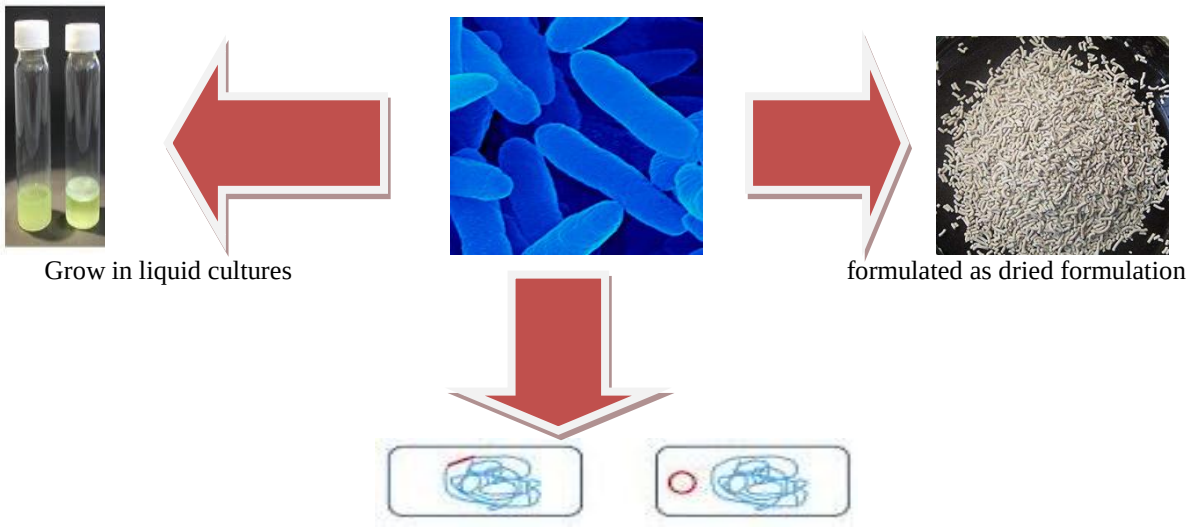


Fig.3:-Characteristics of bacteria as potential bioherbicides

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 nameCamperico (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:-

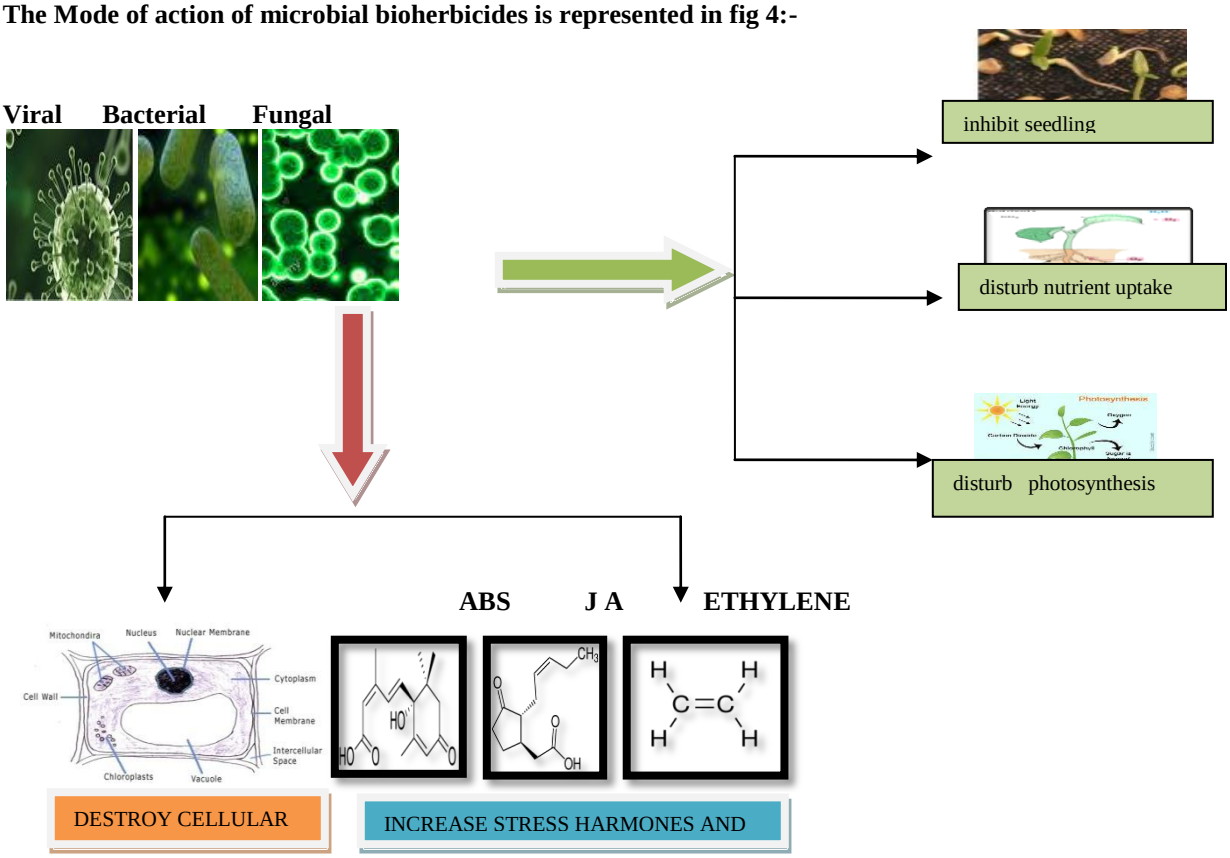


Fig 4:-mode of action of bioherbicides

Plants asbioherbicides:-

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.

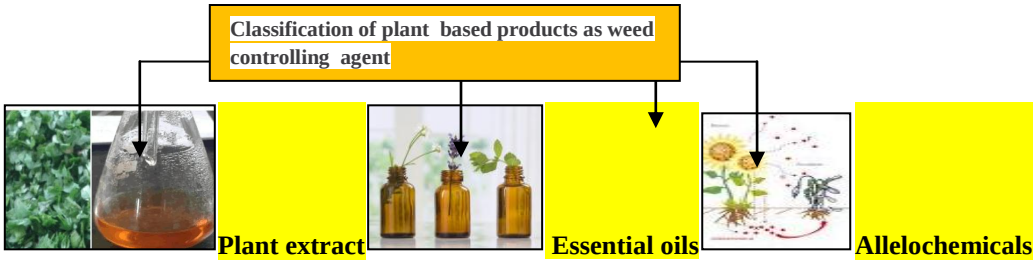


Fig.5:-classifiacation of plant weed

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, secoundary metabolites - alkaloids ,terpenoids ,tetraterpenoids etc.

Table 4:-Plant as bio-herbicides

Plants	**Plant based bioherbicides**	**Mode of action**	**References**
	Plant extracts		
Aglaiaodorata	Leaf extract	Inhibit growth of weed plant	Kato-Noguchi et al., 2016
Usnearoccellina, Everniastrumsorocheilum, andCladoniaconfusa.	methanolic extracts	inhibited root growth and germination of red clover (*Trifoliumpratense*)	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 (Juglansnigra) 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
Cymbopogoncitratus	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 cholorosis 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 (Rosmarinusofficinalis L.), Lawson cypress (Chamaecyparislowsoniana) and White cedar (Thujaoccidentalis) plants	Essential oil	Amaranth, Purslane and Knapwee germination inhibitors species	SadrollahRamezani et.al 2008

Ocimumbasilicum, Menthaspicata, Artemisia vulgaris, Salvia officinalis, Thymbraspicata subsp. spicata)	Essential oil	Inhibit seed germination and seedling growth of eight weed species belongs to different families (Chenopodium album, Agrostemmagithago, Cardariadraba, amaranth , Reseda lutea, Echinochloa crus-galli, Rumexcrispus, Trifoliumpratense).	Onen et al. (2002)
Leptospermum scoparium.	Essential oil	Inhibit seedlings growth	Dayan et.al 2011

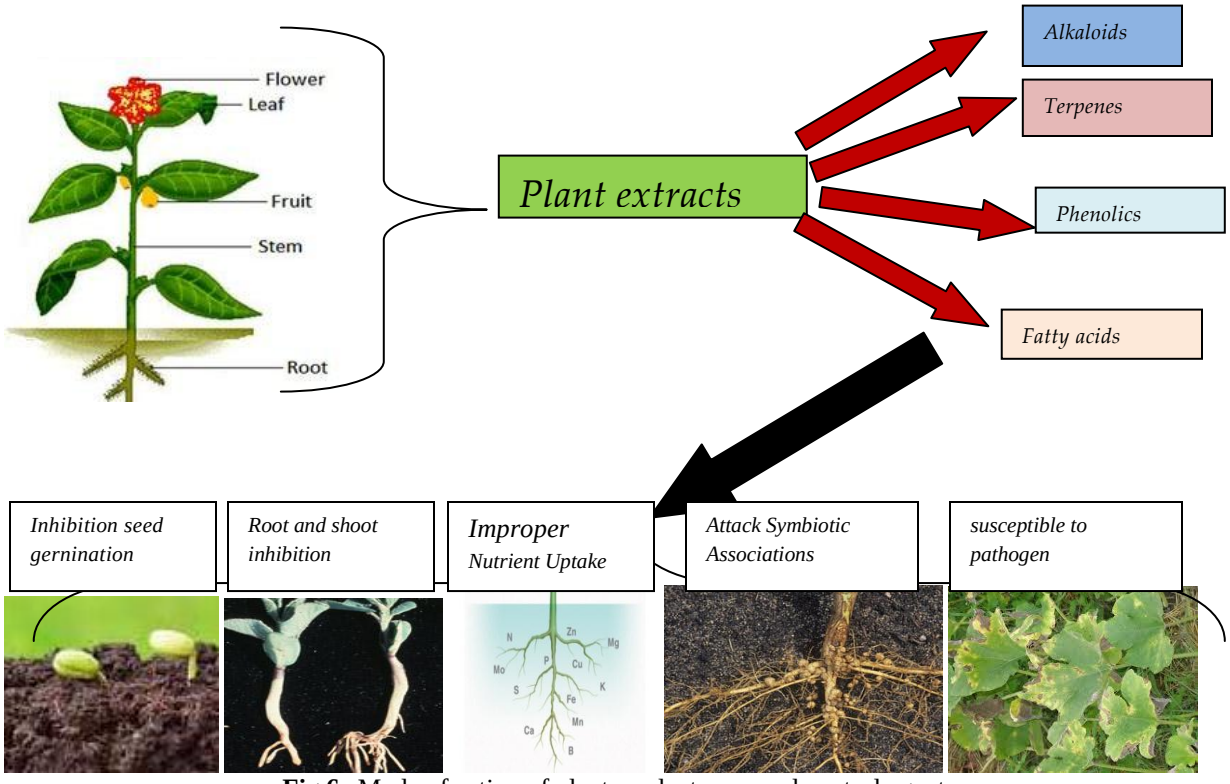


Fig.6:-Mode of action of plant products as weed control agents

Table 5:-Registered pathogens for weed control

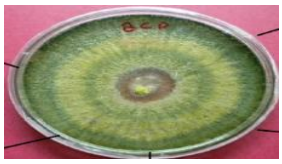
Registered bioherbicides	**Pathogen name**	**Targeted Weed**
Devine [TM]	*Phytophthorapalmivora*	*Morreniaodorata*
Collego TM(1982)	*Colletotrichum gloeosporiodes*	*Aeschynomenvirginica*
Biomal[R]	*Collectrotrichumgloeosporiodes*	*Malvapusilla*
Wood Warrior [R]	*Pucciniathlaspeos*	*Isatistinctoria*
Mycotech[TM]	*Chondrosterempurpureum*	*PrunosSerotine Populous euramericana*
Smoulder[R]	*Sclerotinia minor*	Dicot weeds

Comperico	*XanthomonasCompesteris*	*Poaannoa* L. In turf
Organo –sol [R]	*Lactobacillus casei* *Rhaminouslactis sp.*	*TrifoliumrepensL.* *Trifolium pretense.* *Lotus corniculah L.* *Medicagolupulina* *Oralisacetolla L.*
Pselouka[R]	*Rape seed oil nonanoic acid* *Pelargonic acid*	Weeds of potatoes,grapewmes
Chontrol[TM] Ecoclear[TM]	*Chondrosterium* *Perpureum*	Alders and other hardwoods in forest
Dr.biosedge	*Pucciniacaniculata*	*Cyperusesculantus*
Slumpout[TM]	*Cylindrobasidium*leave	*Acacia Sp.*

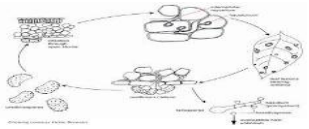
Steps involved in registration of pathogenic bio-herbicides:-

Step1:-Feild survey for pathogenns

Step 2:-isolation of pathogens



Step 3:-mass production of inoculums



Step 4:-Determination of disease cycle

Step5:-formulation development

Step6:-trails in green house for toxicity

Step7. Field trials

Step 8.Registration

Fig.6:-Registration of bio-herbicides

Bioherbicide registeration is the complex as compare to synthetic herbicides. Screening of microbial pathogens and phytoxins 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 (Bo¨ger and Sandmann 1989).This advance approach resulting some improved products that save the time, cost and labour.

Constraints of Pathogens as Bio-herbicide:-

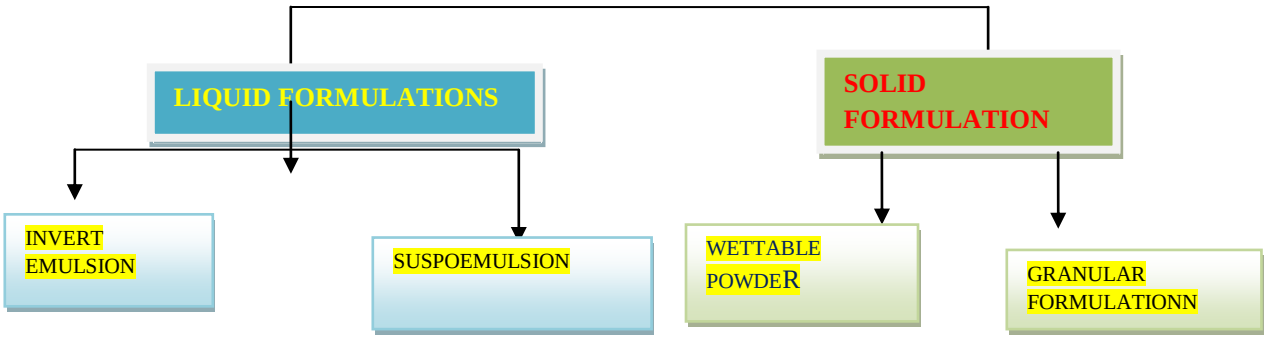
There are many limitations of using pathogens as bio-herbicide such as environmental ,biological, economic or commercial constraints and technological.

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:- • Moisture (dew) (Makowski 1993) • Bioherbicide application time • Formulations • UV radiations, Humidity, and rain water (Leathers et al. 1993). • compatibility with synthetic agrochemicals	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 concide with the required factors for retaintion of water for weed host pathogenecity(Makowski and Mortensen 1990)
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

phytotoxins.	

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
Fusariumoxysporum	Encapsulated granular formulation	Elzein et al.;2004
Fusarium spp.	Encapsulated granular	Amsellem et al.;1999
Fusariumoxysporum	Gum arabic seed coating	Elzein et al.;2006
Myrotheciumverrucaria	Inverted emulsion formulation	Yang and Jong ;1995
Colletotrichumorbicularae	Microemulsion	Klein *et al*. ;1995
Collego and BioMal	Wettable powder	Boyette *et al*.;1996
Alterneriamacrospora	Granular formulation	Walker ;1981
Phomopsissp	Encapsulation of hyphal fragments	Chittick et al. ;2003
AlternariaEichhorneae	Aqueous Mycelial Inoculum	Shabana et al. ;1997
Colletotrichumorbiculare	water suspension	Chittick and Auld ;2001
Exserohilummonoceras	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.