



## A Review on Exploring the Weed Suppressing Characteristics of *Arbuscular mycorrhizal* Fungi for Enhanced Plant Yield and Productivity

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**Abstract:** Weeds are plants, grown out of place, which compete with major crop plants for food, space and light, etc. Various physical, cultural, biological and chemical methods have been used for weed control but they have not been successful mainly, due to increased cost, labour and time. The application of herbicides for weed control has proven helpful but cannot be applied in case of organic farming, which is chemical-free-farming system. Biocontrol methods, utilizing biological organisms, have the potential to control weeds growth to a large extent with limited cost but it has not been well explored. In this method, soil microbes, such as rhizobacteria, pathogenic fungi and root symbiotic fungi, i.e., *Arbuscular mycorrhizal* (AM) fungi have not been widely used. Several studies have shown that AM fungi can inhibit weed growth and proliferation, though the exact mechanism as to how they interact with weed is not known. Reduced seed germination of weeds in the presence of exudates secreted by AM colonized plant roots, changes in relative abundance of mycotrophic (AM host) and non-mycotrophic (non-AM host) weeds species and reduction in weed biomass in response to AM fungi are a few mechanisms reported by various studies till date. The interaction of AM fungi with weeds is not always detrimental but can confer several advantages as discussed in this article. This review article highlights the potential of AM fungi in suppressing weeds growth and intensifies the need to study this mechanism at molecular level to efficiently employ it as weed control method.

**Key words:** Weeds, biocontrol, *Arbuscular mycorrhizal* fungi, enhanced yield and production

### INTRODUCTION

Weeds can be defined as “undesirable plants grown unintentionally and compete with major crops for nutrients, space, light and other required conditions”. It causes significant loss to our major crops’ yield and the loss has been estimated as 34% every year (Oerke, 2006). According to another study, the potential yield loss due to weeds growth could range from 45-95%, subject to various control methods (Ampong-Nyarko and de Datta, 1991). Although, the yield loss can be reduced to large extent, using agricultural practices or toxic chemicals, known as herbicides (Wyse, 1994), but these are expensive, not ecologically and environmentally safe and not always friendly to our major crops. They are also the potential contaminant if leached below ground water and may ultimately enter in our bodies through unfiltered drinking water. Another problem, associated with herbicides application, is that more than 200 weeds have shown strong resistance against most of the herbicides (Heap, 2015). It is because they can confer resistance against various modes of actions of herbicides. Controlling weeds with herbicide application cannot be

accomplished in case of organic farming. It is a kind of farming, where no chemical is added for weed control or getting enhanced crop yield but mostly rely on natural plant material and cultural techniques without harming the environment (Basker, 1992). The good thing in using herbicides for weed control is that it has no negative effect on crop colonization by AM fungi that have widely been noticed in case of mechanical weed control methods presumably due to AM fungal hyphal damages (Brito *et al.*, 2013).

**Biocontrol method for weed control:** Understanding the limitations and hazardous effects of herbicides, the potential of microorganisms for weed control may be exploited (Templeton *et al.*, 1979; Wapshire, 1974). In biocontrol method, which is the application of biological organisms for controlling weeds growth and proliferation, microbes have been used as foliar pathogens. Unfortunately, in this method of weed control, soil microbes, such as, rhizobacteria, pathogenic fungi and root symbiont arbuscular mycorrhizal fungi, have not been exploited. Later studies reported that these microbes can affect growth and development of weeds directly or

indirectly, which show the potential of these microbes for weeds control (Boyetchko, 1996). Foliar pathogen application can only be successful, if these microbes come in contact with shoots (leaves or stem), which is a prerequisite for infection to occur. It may not be successful in case of weeds, where meri-stem is protected with leaf sheath and not possible for fungus to reach weed stem (Greaves and MacQueen, 1991). Considering this problem, efforts shall be directed towards soil and seed borne microbes. The obvious problem, related to soil microbes, is complexity in their inoculation and delivering techniques. Another complexity, associated with biocontrol of weeds, is choosing and screening of single plants for identifying potential microbe, which can reduce or inhibit weed growth. Such screening of single plants for potential microbes is time consuming, laborious and not natural (Boyetchko, 1996).

In addition to biocontrol of weeds, some tissue culture based practices utilizing cell and callus culture for monitoring rhizobacteria were established for biocontrol of weeds (Souissi and Kremer, 1994). Despite knowing the presence of diverse microorganism in soil rhizosphere and their potential as weed control, these microbes have often been ignored by earlier studies. Now, the need is to properly explore soil microbes, their community structure, evaluate different strains having potential for weed control and examine the ecology of rhizosphere and microbial community (Boyetchko, 1996; Corneo *et al.*, 2013). The interaction of weeds and soil microbes has been studied both *in vivo* and *in vitro* using mostly bacteria and in a few cases arbuscular mycorrhizal fungi. These studies have shown that weeds affect soil microbial composition (Carson *et al.*, 2007; Corneo *et al.*, 2011). Similarly, some weeds have been reported to have its specific microbial community in the soil (Sarathchandra *et al.*, 1997).

This review article focuses, how AM fungal colonization reduces the weed growth, is there any grading or differences in reducing weed growth if in association with single or combination of AM fungal species? Is there any increase or decrease in weed plants growth in the presence of a crop species?

**Significance of the study:** Various methods have been used for weed control, including physical, cultural, mechanical and chemical control methods. These weed control methods have not been successful, due to some limitations. Mechanical weed control methods can disturb soil microbial community composition/structure and may have negative effects on crop plants colonization by AM fungi presumably due to fungal hyphal damages. In addition to possible hazardous effects of chemical/herbicides application on soil microbes and

vegetation, many weeds have shown strong resistance against many herbicides. This study emphasizes the need to manipulate the potential of soil and roots borne microbes, such as AM fungi for suppressing weed plants growth, which has not been noticed by earlier studies. This study also stresses on exploring the weed inhibiting mechanism of AM fungi on molecular level to enable them to be applied on large scale weed control programmes.

#### **Arbuscular mycorrhizal fungi and weed control:**

Arbuscular mycorrhiza is mutualistic association between plants roots and fungi, in which both partners get benefit from each other. The plant hosts provide the fungus with carbohydrates and get many things in return, including enhanced nutrient uptake and tolerance against biotic and abiotic stress, such as drought, salinity and insects, etc. Arbuscular mycorrhizal (AM) fungi can colonize about 85-90% plants, the remaining 10-15% plants are mostly weeds (Jordan *et al.*, 2000). These plants seem to have lost the ability to be colonized by AM fungi presumably by adapting themselves to a particular area and developing deep rooting system for the uptake of necessary nutrients from the soil (Brundrett, 2009; Wang and Qiu, 2006). AM fungi is one of the microbe which has been manipulated for this purpose (Cameron, 2010; Jordan *et al.*, 2000), though not much is known as to how these fungi interact with weeds. One study (Jordan *et al.*, 2000) demonstrates that these fungi may alter the nature and structure of weed communities presumably by altering the relative abundance of mycorrhizal and non-mycorrhizal plants. This study further illustrates that AM fungi do not always adversely affect weeds growth but interaction with weeds reduces the crop yield losses subjected to if weeds minimize their negative effects on crop plants and enhance their positivity towards improving crop yield, soil microbial community structure and soil properties. Cultural practices have been reported to affect the infectivity of these fungi under different cropping system, notably organic and conventional cropping system (Bilalis *et al.*, 2011) (Table 1, 2). A meta-analysis study conducted recently has demonstrated the potential of AM fungi towards weeds growth suppression either directly or indirectly. They can either reduce weeds growth by directly suppressing its growth or by increasing the competitive effects of the major crop plant, i.e., increasing the nutrients uptake of the crop plant and depriving weeds plants from the essential nutrients, such as, N, P, etc. It was also suggested that the relationship of crop-weed-AM fungi should be properly investigated before employing these fungi in integrated weed management programme (Li *et al.*, 2016).

Table 1: Effects of cultural/farming system (Org = organic; Con = conventional) on different parameters of weeds (Bilalis *et al.*, 2011)

Weed type	Weed density (no. m <sup>-2</sup> )		Weed biomass (gm <sup>-2</sup> )		Weed N (%)	
	Org.	Con.	Org.	Con.	Org.	Con.
<b>Non-competitive</b>						
<i>Anthemis arvensis</i>	4.7±0.46	3.1±0.32	14.6±1.12	10.7±0.67	4.54±0.21	4.20±0.12
<i>Fumaria officinalis</i>	3.3±0.42	2.8±0.34	2.2±0.76	3.5±0.87	1.93±0.21	1.24±0.19
<i>Lamium apaleicaule</i>	4.2±0.54	4.7±0.65	9.6±0.43	9.1±0.61	2.94±0.32	2.38±0.12
<i>Lolium rigidum</i>	1.5±0.31	1.3±0.45	7.2±2.10	5.2±1.78	3.56±0.14	2.92±0.32
<i>Stellaria media</i>	3.4±0.12	3.2±0.41	12.3±1.76	10.2±1.54	2.54±0.12	2.59±0.32
<i>Veronica hederifolia</i>	2.2±0.39	2.7±0.41	8.8±0.98	6.9±1.32	4.27±0.31	3.80±0.18
Total <sup>a</sup>	19.3 a	17.8 a	54.1 a	44.5 a	-	-
<b>Competitive</b>						
<i>Avena sterilis</i>	7.2±1.05	3.4±1.32	14.5±0.61	10.5±0.52	4.06±0.45	3.26±0.32
<i>Gallium aparine</i>	3.9±0.32	2.9±0.45	18.9±2.45	8.9±3.21	1.79±0.13	1.29±0.18
<i>Lactuca serriola</i>	4.1±0.21	4.2±0.48	20.6±2.76	11±1.78	3.31±0.12	2.99±0.09
<i>Picris echioides</i>	3.1±0.21	1.9±0.89	13.1±0.79	7.4±1.49	3.56±0.11	3.15±0.14
<i>Plantago lanceolata</i>	3.9±0.63	2.2±0.51	18.8±1.83	9.8±4.14	3.66±0.32	2.92±0.17
<i>Sonchus oleraceus</i>	3.3±0.32	3±0.67	24.3±3.65	14.2±2.77	2.78±0.45	2.51±0.31
Total	25.5 a	17.6 b	110.2 a	61.8 b	-	-

Table 2: Correlation coefficients between various parameters of weeds (Bilalis *et al.*, 2011)

	Organic				Conventional			
	WB	RD	AM	N (%)	WB	RD	AM	N (%)
<b>2008</b>								
Weed density (WD)	ns	ns	ns	ns	ns	ns	ns	ns
Weed biomass (WB)		ns	0.53**	ns		ns	0.47*	ns
Root density (RD)			ns	0.53**			ns	0.64**
AM root colonization				ns				ns
<b>2009</b>								
Weed density (WD)	ns	ns	ns	ns	ns	ns	ns	ns
Weed biomass (WB)		ns	0.67*	ns		ns	0.67*	ns
Root density (RD)			ns	0.61**			ns	0.61**
AM root colonization				ns				ns

\*, \*\*Significant at p = 0.05 and p = 0.01, respectively, ns: Not significant

Table 3: AM fungi as biocontrol agents influencing the growth of parasitic weeds

AMF symbiont	Crop/weed tested	Agroeco system service	Reference
<i>G. mosseae</i> , <i>G. intraradices</i> and <i>G. coronatum</i>	sunflower	Suppressed weed growth	Rinaudo <i>et al.</i> (2010)
<i>Glomus intraradices</i> , <i>Glomus mosseae</i> , <i>Glomus claroideum</i>	<i>Echinochloa crus-galli</i> , <i>Setaria viridis</i> , <i>Solanum nigrum</i>	Weeds suppression	Veiga <i>et al.</i> (2011)
<i>Rhizophagus irregularis</i> , <i>Gigaspora rosea</i>	<i>Orobanchae</i> , <i>Phelipanche</i> spp, witch weeds	Supress weeds seeds germination	Louarn <i>et al.</i> (2012)
<i>R. irregularis</i>	<i>Arabidopsis thaliana</i>	Reduce growth	Veiga <i>et al.</i> (2013)
<i>R. irregularis</i>	<i>Avena sterilis</i> , <i>Fumaria officinalis</i> , <i>Stellaria media</i>	Reduced growth	Bilalis <i>et al.</i> (2011)
AM fungi	Rice	Reduced weeds growth	Adeyemi <i>et al.</i> (2015)

The AM fungi are believed to be ecologically important to several plants (mostly vascular) and they have the potential to alter the ecology of weeds species and can influence their growth without affecting the growth of major crop (Jordan *et al.*, 2000). A study conducted to examine the effects of AM fungi on weeds growth showed that none of the weeds studied got positive growth response with AM fungal association. It has rather shown enhanced negative effects on weeds growth (Fig. 1). After studying the growth response, the authors also demonstrated that the presence of crops further increase the harmful effects of AM fungi on weeds growth (Rinaudo *et al.*, 2010; Veiga *et al.*, 2011;

Adeyemi *et al.*, 2015). A similar experiment was performed by Rinaudo *et al.* (2010), where they grew a crop with six diverse weeds in the presence of AM fungi (Table 3). They reported 47% reduction in weed biomass from test where all weeds were grown together and 25% reduction in weed biomass grown alone. Plant growth was promoted even in the presence of six strong competitors (weeds), which highlight the ability of *Arbuscular mycorrhizal* fungi to cope with diverse growing conditions.

Table 4 shows percentage of AM fungal colonization and plant density during 2011-2012.

“Mono” indicates plant species cultivated alone, “mix” weeds grown in combination with maize. Monocultures and mixture of weeds inoculated with AM fungi (grey bars) or non-AM inoculated (black bars), Asterisks indicate significant differences in total biomass (AM colonized or non-AM). Numerical values (white) indicate mycorrhizal response in mono cultures or mixtures.

Variable effects of AM fungi on the mycorrhizal weeds species have been reported as some weeds species can be more strongly influenced than others. The adverse effects of AM fungi on weeds growth can vary with AM fungal strains and their relative abundance. More strong negative fungal effects on weeds (non-mycorrhizal) growth have been observed in the presence of AM fungal hyphae presumably due to secretion of some substances from the hyphae (Veiga, 2012), but no evidence has been presented so far to support this observation. One study (Fernandez-Aparicio *et al.*, 2010) has demonstrated the reduced seed germination of *Orobanch* and *Phelipanche* under the influence of substances (exudates) secreted from two arbuscular mycorrhizal colonized leguminous plant roots (Fig. 1, 2). These exudates secreted from the root cells could be either of low molecular weight or variable molecular weight and also water soluble (Rovira *et al.*, 1979). The presence of exudates influence microbes in the rhizosphere and have been reported to be the major carbon source for their growth (DeAngelis *et al.*, 2009). Similar findings of reduced seed germination under the influence of AM fungi have also been demonstrated by several studies which show the potential of these fungi as key biocontrol agents (Louarn *et al.*, 2012; Lendzemo *et al.*, 2005, 2006;

Hearne, 2009; Gworgwor and Weber, 2003; Sui *et al.*, 2014). Other studies (Malik and Charaya, 2012a, b) have reported 85 and 100% reduction in seed germination of

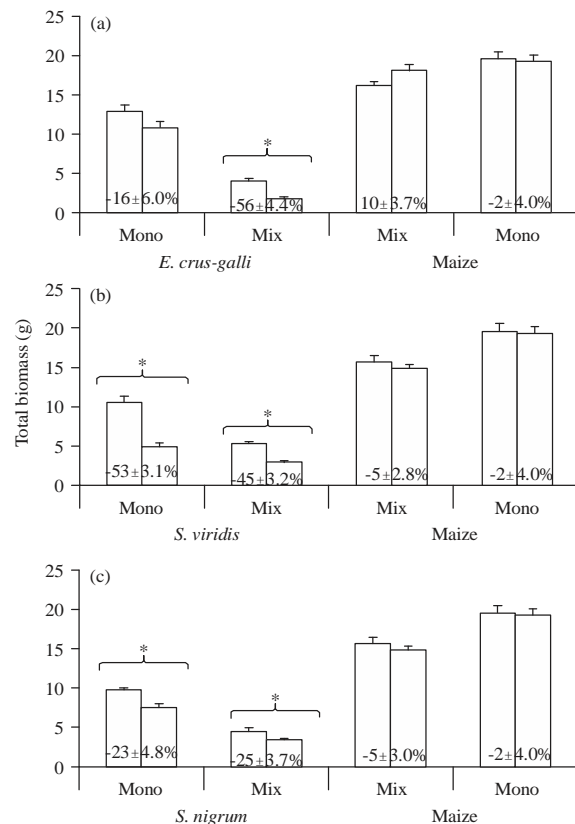


Fig. 1(a-c): Total biomass of three weeds (*E. crus galli*, *S. viridis*, *S. nigrum*) and maize (Veiga *et al.*, 2011)

Table 4: Percentage of AM fungal colonization and its spore abundance affected by rice varieties and plant density during 2011-2012 (Adeyemi *et al.*, 2015)

Variety (V)	Density (D) (Plants ha <sup>-1</sup> )	AM spore abundance			AM colonization		
		2011	2012	Mean	2011	2012	Mean
ITA 150	333,333	40.00	15.67	27.84	63.3	25.33	44.32
	250,000	24.30	3.67	13.99	78.9	40.67	59.79
	166,666	9.30	7.33	8.32	83.3	56.0	69.65
	Mean	24.54	8.89	16.72	75.17	40.67	57.92
NERICA II	333,333	28.70	6.33	17.52	60.00	41.33	50.67
	250,000	35.30	5.0	20.15	51.1	32.00	41.55
	166,666	29.70	7.33	18.52	61.1	54.67	57.89
	Mean	31.23	6.22	18.73	57.4	42.67	50.04
OFADA	333,333	31.70	13.33	22.52	76.70	42.67	59.69
	250,000	17.00	9.00	13.00	62.20	44.00	53.1
	166,666	20.00	31.33	25.67	81.10	33.33	57.22
	Mean	22.9	17.89	20.40	73.33	40.00	56.67
SED (±)							
Variety (Y)		ns	*		7.06*	ns	
Density (D)		ns	*		ns	ns	
V×D		ns	*		ns	ns	

ns: Not significant, \*Significant at p<0.05

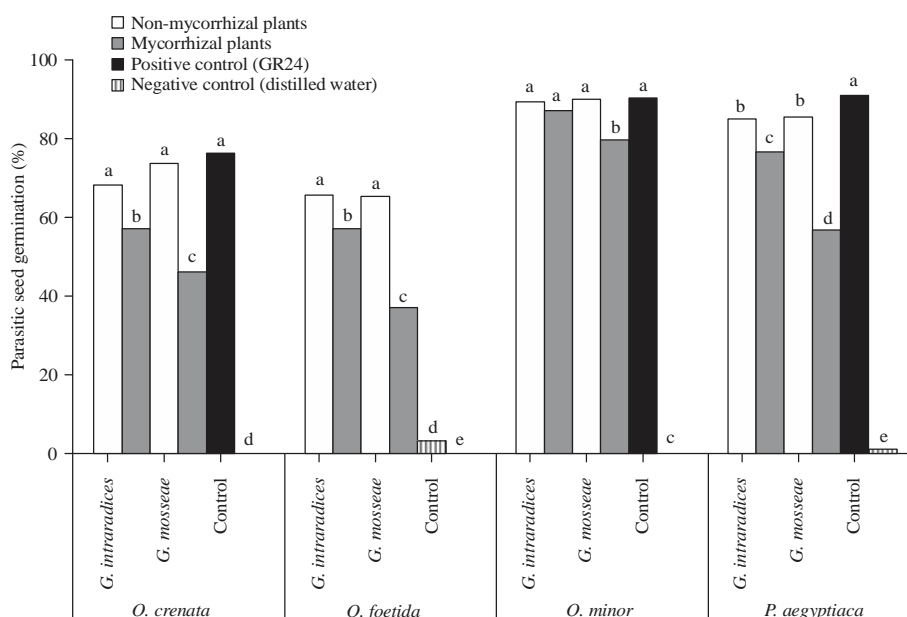


Fig. 2: Percent seeds germination of *Orobanche crenata*, *O. foetida*, *O. minor* and *Phelipanche* in response to plant root exudates which have been colonised by *G. intraradices* (*G.i.*) and *G. mosseae* (*G.m.*) and control plants (non-mycorrhizal), (taken from Fernandez-Aparicio *et al.*, 2010)

parthenium (weed) in response to culture filtrate of *verticillium* species and *Aspergillus flavus* respectively. The reduction in seed germination was observed in late stages which is preferred in the earlier stages for efficient biocontrol of weeds. Sometimes, the effect of AM fungal treatments is less effective but the good thing is that it is normally long lasting in population (Malik, 2011).

Haustorium formation was found to have been inhibited in roots of *Pedicularis tricolor* after being inoculated with arbuscular mycorrhizal fungi, which shows the potential role of these fungi in the management of *Pedicularis tricolor* and *Pedicularis rex* (Li *et al.*, 2013). However, the exact mechanism by which Haustorium formation has been suppressed by AM fungi is still unknown. Some studies have demonstrated dense and extensive growth of weed roots in crop area heavily infected with weeds. These roots have been observed to secrete some substances, which support weeds growth but reduce or inhibit crop growth. The effects of these root exudates on soil microbes have also been reported which support only specific soil dwelling microbes. AM fungi which promote the nutrient uptake of plants have been demonstrated to be adversely affected with the presence of these root exudates. Such adverse effects on AM fungi have mostly been reported from the herbicide resistant weeds, which disrupt the symbiotic relationship of plants and AM fungi (Kremer, 2014).

It has also been reported that AM fungi encourage the growth of mycorrhizal plants but inhibit the growth of non-mycorrhizal plants (Allen and Allen, 1984). One of the approaches used by AM fungi in inhibiting weed growth is changing the relative abundance of AM-colonized-weeds and non-AM-colonized weeds species in agroecosystem (Jordan *et al.*, 2000). Plants species that do not form mutualistic relationship with AM fungi mostly belong to non-mycotrophic (not AM host) (Newman and Reddell, 1987) species. One reason for lack of mutualistic association in such cases may be incompatibility between plants and AM fungi, which may be indicated by browning of the plant roots, which would not have been seen if there is compatibility between plant roots and AM fungi. Plant roots after getting brown, cannot be infected by these invading fungi, the browning effect of the roots have been reported to be because of phenol accumulation in roots (Anderson, 1988).

The interaction of weed species with AM fungi is not always negative as some weed species have been reported to influence the diversity and abundance of agronomically important arbuscular mycorrhizal fungi (Vatovec *et al.*, 2005). Such positive interaction of weeds and AM fungi has resulted in reduced losses of crop yield, arising from weeds and has shown positive effects of weeds on soil properties and community composition of soil microbes. Similarly a positive correlation has been observed

between weeds dry weight and AMF percentage under the field condition. In such cases, the presence of a particular weed acts as determinants of a particular AM fungi (Kubota *et al.*, 2015).

The AM fungi residing in the weeds free cultivated soil have been shown to be less diverse and efficient in supplying nutrients to the host plants as compared to soil containing weed species. They can also improve the mutualistic symbiosis with agronomically important crops (Feldmann and Boyle, 1999) which have resulted in enhanced AMF colonization of several crops (Kabir and Koide, 2000; Sorensen *et al.*, 2005). Another study reporting the positive relationship of AM fungi and weeds biomass has demonstrated significant increase in weed biomass (Kubota *et al.*, 2014). AM fungi can sometimes act as pathogen when soil conditions are not favourable, i.e., genes responsible for pathogenicity can be turned on under adverse soil condition, such as, phosphorous deficiency (Ryan *et al.*, 2005). Similarly, not all weeds growth can be negatively affected by all AM fungi; therefore, they cannot be employed as efficient biocontrol system (Veiga *et al.*, 2012; Sui *et al.*, 2014). Some studies (Chen *et al.*, 2004) have reported increased number of arbuscular mycorrhizal fungal spores when studied in association to large number of weeds species and enhanced positive effects of AM fungi on the growth and existence of *Vincetoxicum rossicum* species (Smith *et al.*, 2008).

**Limitations of AMF assisted weed biocontrol programme:** Although AM fungi have played significant role in weed control and increased crop yield and productivity to a large extent but it has not achieved the target, due to following constraints:

- Several strains of AM fungi have been reported which respond differently (Bilalis *et al.*, 2011) towards weed suppression. It is difficult to identify which fungal strain is efficient in suppressing weed growth due to lack of enough knowledge about genetics and genetic diversity of these fungi
- Some weed plants have been found to secrete exudates, which encourage weed growth but inhibit the growth of major crops. AM fungi have been reported to be adversely affected with the presence of these root exudates. Such adverse effects on AM fungi have mostly been reported from the herbicide resistant weeds which disturb the symbiotic relationship of plants and AM fungi
- The germination of some weed seeds have been inhibited by exudates secreted by AMF colonized plant roots. Reduction in weed seeds germination was observed at a later stage, which should ideally be in the first stage for efficient weed control programme

## CONCLUSION

Various methods reported previously for weeds control have not been widely applied mainly, due to increase in cost, harmful effects and low efficacy. Biocontrol methods of weed control have not been well explored, which could significantly reduce the loss in crop yield, associated with the presence of diverse type of weeds plants. Among the soil microbes, AM fungi have the potential to inhibit or reduce weed plants growth, using a mechanism not been well explained till date. The potential of AM fungi for suppressing weeds growth can be exploited as one of the important biocontrol methods. According to a study conducted on weeds and AM fungi, the more adverse effects of these fungi on weeds growth can be observed in the presence of AM fungal hyphae due to unknown reasons. The interaction of AM fungi with weeds is not always harmful; some studies have shown positive effects of weeds on soil properties and community composition of soil microbes. Similarly, AM fungi, reported from weeds free soil, have shown to be less effective in supplying nutrients to its host plants than soil containing weeds. As, not all strains of AM fungi can inhibit weed plants growth, therefore, proper screening for the identification of efficient fungal strains is a prerequisite for a successful biocontrol programme.

As chemical control of weeds utilizing herbicides is expensive, ecologically and environmentally unsafe, efforts shall be directed towards biocontrol of weeds exploiting the potential of AM fungi for weed control. All potential strains of AM fungi should be isolated, cultured *in vitro* and tested for their ability for weed control programme. The potential of these fungi for weed control shall be monitored regularly in order to retain the efficient strains only. Only those fungal strains shall be included in weed control programme, which have been proved effective against most of the weed species. The exact mechanism is that how these fungi inhibit weed growth shall be studied at molecular level in order to identify the sites for improvement. Considering these recommendation, it is possible to suppress weeds growth to large extent and minimize the yield losses arising from weeds infestations in near future.

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