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Bio-control technology: Development, production and popularization for plant disease control in semi-arid region of Rajasthan, India- A success story

R B GAUR AND R N SHARMA
Department of Plant Pathology, Agricultural Research Station (S.K. Rajasthan Agricultural University), Sriganganagar-335 001, Rajasthan, India
E mail: dr.rbgaur @ yahoo.com

ABSTRACT

The first bio-agent production unit of the state was established at this research station in 1995, funded by the DBT, Govt. of India. Initially exotic isolate of Trichoderma harzianum (Th-8) was used for mass production but latter on a potent native strain of T. harzianum (TG-1) was substituted for commercial production of bio-agent. Systematic studies (1995-2010) were conducted to evolve several biocontrol technologies like, suitable substrates for Trichoderma multiplication, shelf-life of talc based formulation of Trichoderma, delivery system of antagonists to seed, soil and paint formulation, dry root rot control in chickpea using Khejri based production system, development of new consortium formulation of bioagents and compatibility of bio-agents with fungicides. Several recommendations were provided for successful biocontrol of plan diseases like root rot of chickpea and cotton (Rhizoctonia bataticola), Sclerotinia stem rot of mustard (Sclerotinia sclerotiorum), Phytophthora rot/gummosis (Phytophthora spp.), canker (Xanthomonas axonopodis pv. Citri) and fruit dropping in Kinnow. These technologies were transferred to cultivators systematically through front line, field and IPM based demonstration. Since establishment of bioagent production unit, a total of 9855 kg of talc based Trichoderma has been produced for commercial sale and income of Rs. 11,69,340/- has been generated. This successful transfer of bioagent technology has led to generate interest of State Agricultural Department and private companies in the market to meet out the growing demand of bioagent in the area. The commercial sale through these sources in this zone between 2007-08 to 2009-10 was approximately 87 metric tonnes. During this 15 year of journey on eco-friendly road approximately 13,633 ha and 1,23,142 ha area has been covered under this technology through commercial sale of bioagent by this centre and Govt. and private agencies respectively. This clearly indicates impact of technology generated by this centre in managing soil borne diseases of the area and improving soil health.

Key words: Bio-control, Trichoderma, crops.

In intensive agriculture pesticides have been looked upon as omnipotent weapons for modern disease management. Indiscriminate use of pesticides against crop diseases has created several problems related with environmental pollution, development of resistance in pathogen and accumulation of harmful residue in soil, water and plants. This has prompted the necessity for development of alternative that could be practical and effective for eco-friendly disease management. Steady efforts were therefore made to develop cost effective, eco-friendly, viable bioagent based technology to manage important soil borne plant pathogens such as *Phytophthora*, *Rhizoctonia*, *Fusarium*, *Sclerotinia* which have detrimental effect on kinnow, cotton, chickpea and mustard crop of economic importance in the region.

Establishment of bioagent production unit

Keeping this in view, a mission mode pilot project on "Development, production and demonstration of bio-control agents under integrated pest management" was submitted by R.K. Joshi and S. Gangopadhyay. A bioagent production unit was finally established in 1995 under the funding (Rs. 33 lac) of DBT, Government of India for production and demonstration of bioagents for the management of pests and diseases in cotton and chickpea crops in semi-arid region of Rajasthan. This was the first bioagent based lab in the State.

Preliminary studies

Collection of bioagent isolates & mass production of *Trichoderma*. Preliminary bioagent based studies (1995-1998) were conducted by Gangopadhyay and Joshi. Initially, different isolates of bioagents were collected from biocontrol labs of Bangalore, Coimbatore and Pantnagar to select the suitable strains for the region. Exotic isolate of *Trichoderma harzianum* (Th-8) obtained from Pantnagar was used for mass production of bioaged for field demonstration and trials.

Field demonstrations and trials. Large scale multilocational field demonstrations were conducted in farmers' fields and Central State Farm, Suratgarh in cotton and chickpea crops in the fields having known history of root rot infestations. Seed treatment with Trichoderma-SD @ 4g kg^{-1} seed (talc-based formulation of 2 x 10^7 cfu g^{-1}) plus soil application of Trichoderma SD @ 5 kg ha^{-1} were found effective in controlling root rot in cotton and chickpea crops (Gangopadhyay and Joshi, 1997). This was the first bioagent based recommendation in the zone.

Development and refinement of technology

Potent bioagent isolate of *Trichoderma.* Work on refinement of bioagent based technology was initiated during 1998 by Gaur and Sharma. Collection of soil samples from the zone -1b (Sriganganagar and Hanumangarh district) of Rajasthan were made to

isolate potent *Trichoderma* strains among 13 local isolates. Thereby during 1999, the commercial production of *T. harzianum* (Th-8) in bioagent production lab was substituted with potent local strain (TG-1) of *T. harzianum* (Gaur and Sharma, 1999).

Suitable substrates for *Trichoderma* multiplication. Ten substrates viz., wheat bran + 2% molasses, wheat bran + peat soil (3 : 10), Sugarcane bagasse + 2% molasses, gram shells, paddy husk, sorghum grains, sodium alginate granules impregnated with 2% molasses, farm yard manure (FYM), faecal pellets of goat & sheep, standard talk based formulation were evaluated for *Trichoderma* multiplication. Faecal pellets of goat and sheep, gram shell and FYM were identified as most suitable substrates for *Trichoderma* multiplication. Sorghum grain substrate of *Trichoderma* was found least suitable for bioagent multiplication (Gaur *et al*., 2005a).

Shelf-life of talc based formulation of *Trichoderma.* The viability of *Trichoderma* in talc based powder formulation was estimated (1999-2000) at different storage temperature ranging between 0 to 40 ^{o}C for 180 days of storage (DOS). The initial mean population of *Trichoderma* on 0 day was 270 x 10^6 cfu $g^{-1}$and after 180 DOS it declined to 20 x10^6 cfu g^{-1}. Shelf - life of 90-105 days at 25 ^{o}C was worked out for commercial produce of *Trichoderma* (Gaur *et al*., 2005a).

Delivery system of antagonist to seed and soil. The delivery system of antagonist to seed & soil was worked out during 1999-2000 to 2002-2003. Technique of soil application of *Trichoderma* was found significantly superior over seed treatment. Pre-incubation of *Trichoderma* in FYM 15 days before soil application gave better results than its alone application. Wheat bran based formulation of *Trichoderma* gave better control of disease than talc based formulation for soil application. *Trichoderma* @ 10 g kg^{-1} seed gave better control than the lower dose of 4 g kg^{-1} seed. Technique of seed dip, solid matrix priming did not prove effective (Gaur *et al*., 2005b). Seed treatment involving single bioagent with or without spray combination though provided significantly better control of disease in comparison with control but were found inferior when compared with mixture of two different bioagents (Gaur *et al*., 2010).

Delivery system of antagonist for paint formulation. The delivery system of antagonist for paint formulation was also worked out (2004-05 to 2006-07) to find out environmentally accepted paint formulation, alternative to metalaxyl for the control of foot rot disease in kinnow. Different paint formulations of *Trichoderma* and *Pseudomonas* were explored against *Phytophthora* stem lesions in Kinnow Painting with talc based formulation of *P. fluorescens* (2 x 109 cfu/g) or *T. harzianum* (2 x 107 cfu/g) @ 100 g/l water followed by painting with linseed oil after 5 days provided significant recovery of *Phytophthora* lesion (Gaur *et al*., 2011).

Biocontrol agents isolated from different production system. Various types of biocontrol agents (mycoparasites) were isolated (2001-02 to 2002-03) from field rhizospheric soil collected from different production systems in the region in association with various pathogens (Gaur, 2004). Identity was confirmed by Indian Type Culture Collection, IARI, New Delhi and Agharkar Research Institute, Pune.

(a) Khejri based production system. Soil samples collected below khejri (*Prosopis cineraria*) invariably yielded higher bioagent population. Among various species of *Trichoderma* isolates (*harzianum, viride, koningii, hamatum*), *T. harzianum* and *T. viride* were found to be widely distributed while *T. koningii* was found least distributed in soil. Other bioagents isolated from khejri based production system in the region were *Gliocladium* (*virens, penicelloides*), *Chaetomium globosum,* and *Penicillium* (*pinophilum, funiculosum*). Ber/Khejri + Chickpea production system harboured maximum Mycoparasites.

(b) Kinnow based production system. Fruit bearing Kinnow orchards harboured more bioagents and soil borne pathogens compared to pre fruit bearing orchards. The *Aspergillus* group (*versicolor, fumigatus, candidus, flavus, terreus*) was predominant during kherif season when the mung and moth crop was taken as intercrop. *Trichoderma harzianum* was found widely distributed while *Chaetomium globosum* was found least distributed mycoparasites in soil.

Less dry root rot in chickpea under Khejri based production system. Studies were conducted at cultivator's field. Chickpea crop grown under khejri (*Prosopis cineraria*) based production system and as a sole crop was observed during 2001-2002 to 2002-2003. During both the years, 4 fields each measuring 1 ha, was observed for dry root rot incidence. Dry root rot (*R. bataticola*) incidence was significantly less in chickpea under khejri based production system compared to sole crop of chickpea (Gaur, 2004).

Development of new consortium formulation of bioagents. Systematic studies in laboratory, cage house and multilocational field trials were conducted (2002-03 to 2005-06) to find out the best consortium formulation of bioagents for controlling Sclerotinia rot (*Sclerotinia sclerotiorum*) of Indian mustard (*Brassica juncea*). Talc based consortium formulation (2 x 10^7 cfu g^{-1}) of *T. hamatum* (HP-20) + *T. viride* (Tv-1) in the ratio of 1 : 1 (w/w) was developed & commercialized (Gaur and Sharma, 2006).

Isolation of new yeast bioagent. A new yeast bioagent *Sporidiobolus pararoseus* (KFY-1) was isolated from Kinnow fruit surface (Sharma *et al*., 2008b). This bioagent was found effective in controlling pre- and post-harvest rotting of Kinnow fruits (Sharma *et al*., 2008a; Gaur, 2009).

Compatibility of bioagents with fungicides. Fungicides which are compatible with potential bioagents need to be identified for integration in disease management strategies. The compatibility of

potential bioagents with recommended and newly developed fungicides was therefore investigated during the year 2009. Metalaxyl, fosetyl-Al, mancozeb, cymoxanil 8% + mancozeb 64% mixture and copper oxychloride fungicides were found compatible with bioagent *T. harzianum* (TG-1) and tolerance limits (ED_{50}) of these fungicides were >1000 µg/ml. Metalaxyl proved their compatibility with another potential bioagent *T. viride* also where even >1000 µg/ml concentration was under safe tolerance limit (ED_{50}). Copper oxychloride, mancozeb, fosetyl-Al and cymoxanil 8% + mancozeb 64% mixture fungicides showed moderate to good compatibility with this bioagent (Gaur and Sharma, 2011).

Recommendations provided for Package and Practices

Various bio control based recommendations for plant disease management were provided for package and practices of Zone -1b (Sriganganagar and Hanumangarh districts) of Rajasthan during last ten years (2001-2010). Management of plant diseases viz., dry root rot of chickpea and cotton (*Rhizoctonia bataticola*), Sclerotinia stem rot of mustard (*Sclerotinia sclerotiorum*) and *Phytophthora* rot/gummosis (*Phytophthora* spp.), canker *(Xanthomonas axonopodis pv. citri)* and fruit dropping and post-harvest fruit decay in Kinnow was recommended through use of locally developed formulations of fungal bioagent *Trichoderma* (2 x 10^7 cfu g^{-1}), bacterial bioagent *Pseudomonas fluorescens* (1 x 10^9 cfu g^{-1}) or yeast bioagent *Sporidiobolus pararoseus* (10^9 cfu ml^{-1}).

Kinnow mandarin (*Citrus reticulata* Blanco.)

***Phytophthora* rot.** Integrated disease management studies on *Phytophthora* rot of Kinnow were carried out from 1999 to 2002 and recommendation was provided for its control. Stem painting with Metalaxyl 8% + Mancozeb 64% (Ridomil - MZ 72% WP) @ 20 g/l of linseed oil in association with soil drenching *T. harzianum* @ 60 g $tree^{-1}$ in 30 - 40 lit of water twice in each month of February and August at 15 days interval was recommended for integrated management of *Phytophthora* rot of Kinnow. Besides eco-friendly this treatment gave higher net monitory return (Gaur *et al.*, 2004).

Citrus canker. Studies were conducted (2003-04 to 2005-06) for controlling citrus canker of Kinnow and foliar spray of talc based *P. fluorescens* @ 0.2 per cent during the month of February and August, twice in each month at 15 days interval was recommended for eco friendly management of canker disease in Kinnow (Gaur and Sharma, 2010).

Foot rot/Gummosis *(Phytophthora* spp.). Field studies were conducted (2004-05 to 2006-07) to find out bio rational paint formulation, alternative to metalaxyl for the control of foot rot disease in kinnow. Stem painting with talc based *P. fluorescens* or *T. harzianum* @ 100 g lit^{-1} water subsequently followed by painting with linseed oil after 5 days at two times each during February and August gave significant recovery in trunk lesion size of *Phytophthora* and enhanced fruit yield (Gaur *et al*., 2011).

Pre-harvest dropping and post-harvest rotting of Kinnow fruits. Field and lab studies (2007-08 to 2008-09) were carried out for controlling pre-harvest dropping and post- harvest rotting of Kinnow fruits. Three sprays of locally isolated yeast bioagent, *S. pararoseus* in association with Gibberellic acid @ 20 ppm during the month of April, August and September and two sprays alone during March and October were recommended for bio effective management of these diseases of Kinnow fruits (Gaur, 2009).

Cotton (*Gossypium* spp.)

Dry root rot. Antagonistic behaviour of eighteen different native and exotic isolates of *Trichoderma*, *Gliocladium* and *Chaetomium* species was tested (1997-2002) against root rot (*R. bataticola*) of cotton under *in vitro* and *in vivo* conditions fruits and finally recommendation was provided in 2003 year. *Trichoderma viride*, *Gliocladium virens* and *T. harzianum* proved superior to other antagonists in reducing root rot. Pantnagar isolate of *T. harzianum* which was under commercial production in IPM lab till 2008 and *Chaetomium globosum* proved least effective (Gaur and Sharma, 2002a & b). Multilocation field studies conducted on the basis of preliminary green house results revealed that technique of soil application of *Trichoderma* was found significantly superior over seed treatment in controlling of dry root rot of cotton. *Trichoderma* @ 10 g kg^{-1} seed gave better control than the lower dose of 4 g kg^{-1} seed. Technique of seed dip, solid matrix priming did not prove effective.

Conclusively, for effective and economic management of the dry root rot of cotton, seed treatment with *T. harzianum* @ 10 g kg^{-1} seed and soil application of *T. harzianum* @ 10 kg ha^{-1} pre-incubated in 200 kg of FYM for 10-15 days was recommended (Gaur *et al.*, 2005c).

Chickpea (*Cicer arietinum*)

Dry root rot. Multilocational field studies were conducted (1999-2000 to 2002-03) to evolve best application technique of *T. harzianum* for controlling dry root rot (*R. bataticola*) of chickpea. Conclusively, for effective and economic management of the dry root rot of chickpea, seed treatment with *T. harzianum* @ 10 g kg^{-1} seed and soil application of *T. harzianum* @ 10 kg ha^{-1} pre-incubated in 200 kg of FYM for 10-15 days was recommended. Under rain fed situation seed treatment with Trichoderma in association with soil application of Zn So4 @ 25 kg/ha proved effective (Gaur *et al*., 2005b).

Mustard (*Brassica juncea*)

Sclerotinia stem rot. Systematic studies in laboratory, cage house and multilocational field trials were conducted (2002-03 to 2005-06) to find out the best consortium formulation of bioagents for controlling Sclerotinia stem rot (*S. sclerotiorum*) of Indian mustard. Seed treatment with consortium formulation of *T. hamatum* (HP-20) and *T. viride* (Tv-1) in the ratio of 1 : 1 (w/w) @ 10 g kg^{-1} of seed followed by foliar

spray of same bioagent combination @ 0.2 per cent at 50 DAS was recommended for effective management of disease (Gaur *et al*., 2010).

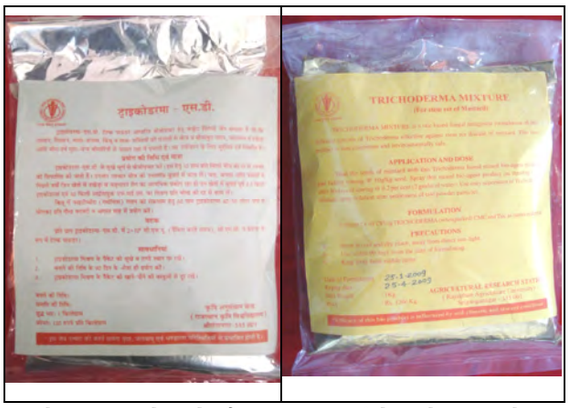
Production of bio agents

This centre has developed three talc based bioagent formulations of *Trichoderma* {Trichoderma SD (Th-8), Trichoderma SD (TG-1), Trichoderma Mixture} for commercial use against soil borne diseases prevalent in the area.

Trichoderma SD (Th-8/TG-1). Production of this talc based formulation (2 x 10^7 cfu g^{-1}) of *T. harzianum* (Th-8) was under taken in 1995 in IPM bioagent production unit setup at this centre under the funding of DBT, Bangalore. During 1998, the commercial production of *T. harzianum* (Th-8) in bioagent production lab was substituted with local strain (TG-1) of *T. harzianum.* This formulation of bioagent is recommended for the control of root rot of cotton, gram, *Phytophthora* rot of kinnow and other soil borne diseases. During the year of 1995 when the production unit was established, the production of this bioagent was only 16 Kg. However in subsequent years, yearly production increased substantially reaching 873 kg in 2009 year. During the period between 1995 and 2009 this unit has produced a total of 8307 kg of Trichoderma SD (Fig. 1).

Trichoderma Mixture. This talc based formulation (2 x 10^7 cfu g^{-1}) of two different strains of *Trichoderma* evolved during 2006 against Sclerotinia stem rot of mustard (Gaur and Sharma 2006). In this formulation, exotic strain of *T. viride*-1 (Tv-1) from Coimbatore and native strain of *T. hamatum* (HP-20) is used in the ratio of 1 : 1. A total of 1548 kg Trichoderma Mixture has been produced at this centre between 2007 and 2009 year (Fig. 1).

Hence since establishment of bioagents production unit, a total of 9855 kg of *Trichoderma* formulations



has been produced for commercial sale to the cultivators and different Govt. and semi Govt. agencies.

Income generation through technology developement

The use of *Trichoderma* for the control of soil born diseases (dry root rot of chickpea and cotton, Sclerotinia stem rot of mustard, Phytophthora rot of Kinnow), a technology developed at the center and recommended for this zone (zone-1b) after duly approval by research and extension wing of university and state, has given substantial source of income generation through direct commercial sale of *Trichoderma* to the cultivators and other Govt. and semi Govt. agencies by IPM production unit. The total income through sale of this product was only Rs. 960=00 when the production unit was established in 1995. However this income reached to the level of Rs. 1,10,760=00 (Rupees One lac ten thousand seven hundred sixty) during the period of 2009. Since the establishment of bio agent production unit, this center has already earned approximately Rs. 11,69,340 = 00 (Rupees Eleven lac sixty nine thousand three hundred forty only) during the period between 1995 and 2009 (Fig. 2).

Popularization and impact of technology

Bioagent use technology for the control of soil borne disease was transferred to cultivators systematically through front line, field and IPM based demonstration. Initially during 1996-98 technology was popularized through large scale (30-32 ha) IPM based demonstrations on cotton and chickpea and the bioagent was distributed among the farmers as a free incentive. Since establishment of bioagents production unit, a total of 9855 kg of *Trichoderma* formulations has been produced for commercial sale to the cultivators and different Govt. and semi Govt. agencies.

This successful transfer of bioagent technology has led to generate interest of State (Rajasthan) Agricultural Department in establishing mini bioagent production unit in this zone. A growing number of private companies have now entered in market to meet out the growing demand of these bioagents. The commercial sale through these alternate sources in the zone between 2007-08 to 2009-10 was approximately 87 metric tonnes (Fig. 5).

Area covered under the technology by research centre. Bioagents based technology developed at this centre has been accepted by the cultivators of this zone comprising Sriganganagar and Hanumangarh district of the Rajasthan. The response was quite encouraging. During the initial year of 1995-96, an approximate area of only 200 hectare was covered under this technology. However, after 15 year of journey on eco-friendly road approximately 13,633 ha area has been covered under this technology through commercial sale of bioagents by this centre (Fig. 3). This total covered area was shared by the prevailing crops like, Kinnow, cotton, chickpea and mustard as 249, 9085, 2423 and 1876 ha respectively (Fig. 4).

Area covered under the technology by Govt. & Private agencies. Beside research centre, an area of about 1,23,142 ha has also been covered through commercial sale by Govt. and private agencies (Fig. 6). Out of this area, 2611, 95156 and 25375 ha was under the crop of Kinnow, cotton and chickpea accordingly. This clearly indicates impact of technology generated by this centre.

This successful transfer of bioagent technology has contributed significantly in managing soil borne diseases of the area and improving soil health.

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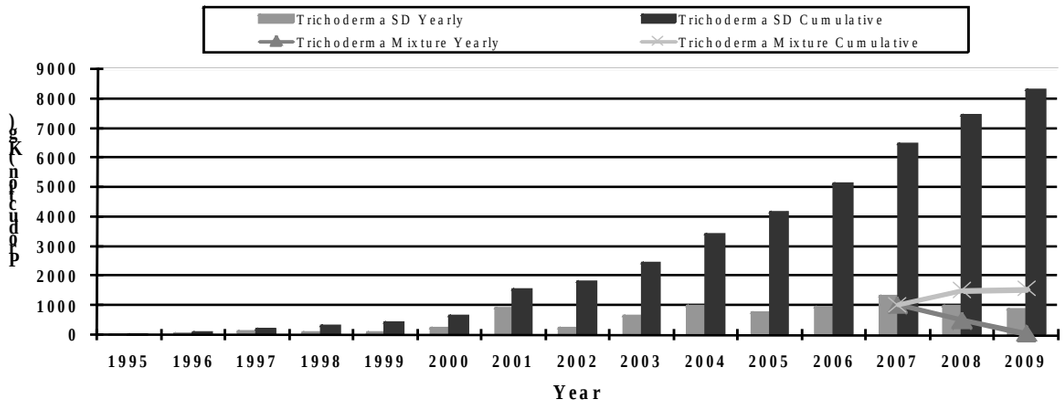

Fig. 1. Commercial production of bioagents at Agricultural Research Station (SKRAU), Sriganganagar (Rajasthan)

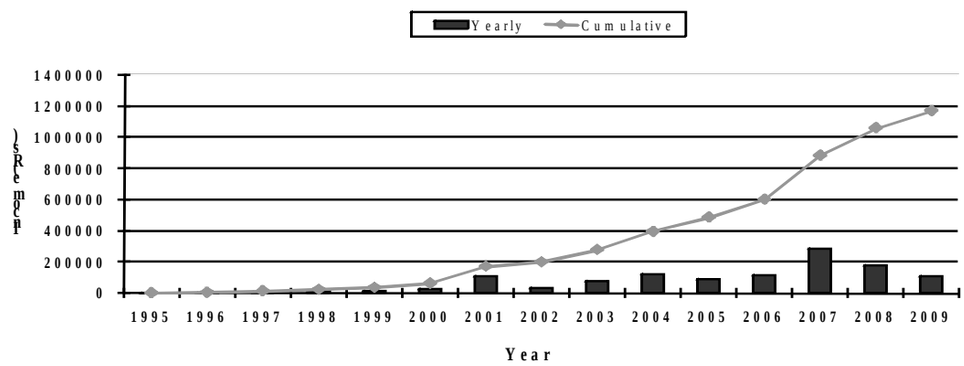

Fig. 2. Income generation through biocontrol technology developed at Research Centre of Sriganganagar (Rajasthan)

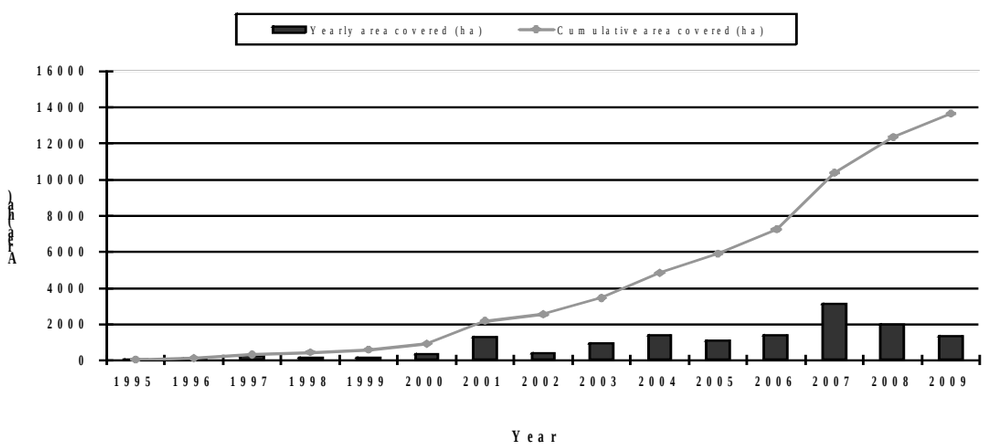

Fig. 3. Area covered under biocontrol technology developed at Research Centre of Sriganganagar (Rajasthan)

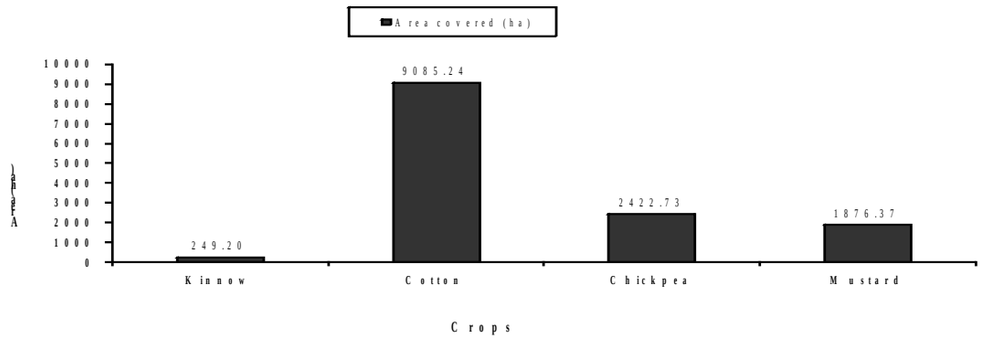

Fig.4. Area covered through biocontrol technology under different crops by Research Centre of Sriganganagar (Rajasthan)

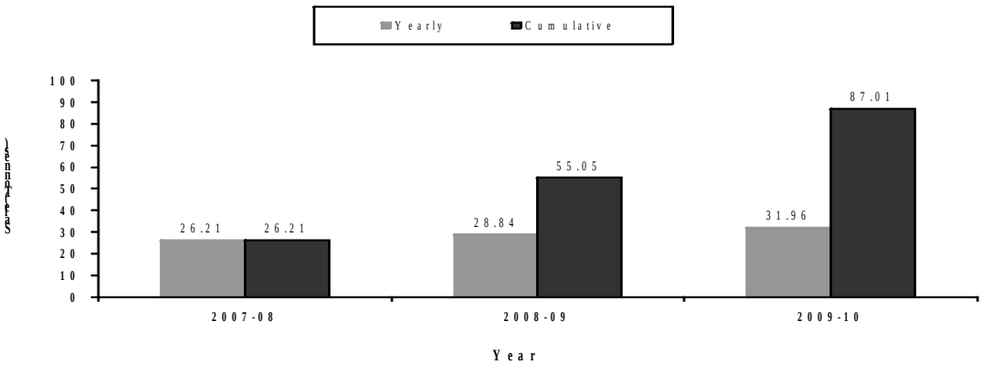

Fig.5. Commercial sale of Trichoderma by private & govt. agencies in Zone 1b of Rajasthan

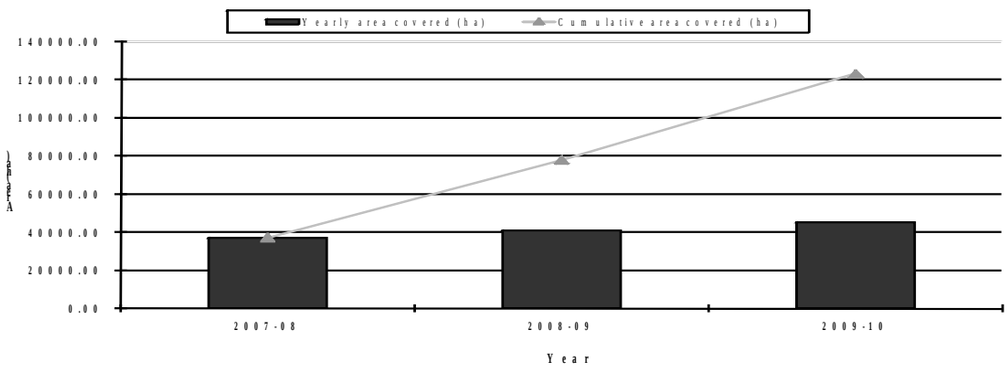

Fig.6. Area covered under biocontrol technology through private & govt. agencies in zone 1b of Rajasthan

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