

Bio-formulations

for disease management

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Under harsh climate of Indian arid region, temperature of top soil layer often reaches 55°C during summer. Resting structures of soil-borne plant pathogens as well as propagules of native bio-control agents bio-control agents (BCA) withstand this temperature. However, there is a need to improve the population of bio-control agents with suitable food substrate so that they can survive and compete well with soil-borne plant pathogens, besides protecting the crops. Efforts were made to explore food substrate for survival and multiplication of bio-control agents to prepare bioformulated products, which can be used by farmers of the region. Effectiveness of bio-formulated products as seed treatment and soil application, were prepared and demonstrated at farmers' field during kharif and rabi. These products were coined as Maru Sena 1, 2, 3 and Mishrit Maru Sena. These demonstrations resulted in wider acceptance by farmers in rainfed and irrigated areas, especially by cumin growers of the region. Every year large number of farmers is purchasing these products from the Institute and thousands of hectare land was brought under cultivation by treating soil and seed for reducing incidence of diseases thereby improving yield of crops.

Key words: *Bacillus firmus*, Cumin, Dry root rot, Legumes, *Macrophomia phaseolina*, *Trichoderma harzianum*, Wilt

Macrophomia phaseolina, a soil borne plant pathogen, causes dry root rot on legumes and oilseeds grown under rainfed conditions. However, considerable variations were observed in disease intensity on these crops even under similar growing conditions. Similarly, cumin suffers heavily due to wilt caused by *Fusarium oxysporum* f. sp. *cumini*, whereas many trees are affected by *Ganoderma lucidum* causing root rot mortality. Several management approaches viz., use of moisture conservation techniques, soil solarization of cruciferous residues, integrating sub-lethal heating with cruciferous residues, composts, weed residues, tolerant genotypes and integrating bio-agents were suggested after considerable research efforts made during past six decades.

There was an upsurge in interest in biological disease control measures recently. This interest was stimulated by the occurrence of fungicide

resistance in some pathogens, and for some soil-borne plant diseases, the lack of reliable chemical controls and availability of disease-resistant varieties. Studies were initiated to explore the potential of biocontrol agents to develop a cost effective and practical management strategy in augmenting disease control. Analysis of large number of soil samples collected from different parts of the region led to isolation of native bio-control agents viz., *Aspergillus versicolor*, *Penicillium oxalicum*, *Trichoderma harzianum* and *Bacillus firmus* from different agricultural systems, which proved their antagonistic ability in laboratory tests.

In the next step, information was required to be generated for their field efficacy on commonly grown crops and their effect on resident microflora to ascertain whether any bioagents showed adverse effect on native micro-organisms.

Native fungal and bacterial bioagents

These bioagents are *Trichoderma*, *Aspergillus versicolor*, and *Bacillus firmus*.

Trichoderma: A heat tolerant strain of *Trichoderma* was isolated from arid soils, which was found effective against pathogens like *M. phaseolina*, *Fusarium oxysporum* f. sp. *cumini* and *Ganoderma lucidum*. These research showed that population of *Trichoderma harzianum* increased gradually in compost prepared from residues of *Prosopis juliflora*, *Calotropis* and weeds compared to farmyard manure and non-amended controls.

Aspergillus versicolor: During the course of these investigations, it was frequently observed that population of one species of *Aspergillus* increased tremendously in residue amended heated soil. When separate laboratory tests were performed, it was observed that this strain was parasitizing *Fusarium* sp. propagules as well as sclerotia of *M. phaseolina*.

Subsequently, detailed studies were initiated to confirm the bio-control potential of this fungus, which was identified as *A. versicolor*. Studies related to thermal resistance of *A. versicolor* showed that it was able to survive and multiply even at 65°C. A positive correlation was observed between time interval and increase in population of *A. versicolor*.

Bacillus firmus: In the recent past, a novel bacterium, *B. firmus* was isolated from naturally heated cruciferous residue amended soil that showed antagonistic activity against *M. phaseolina*. In dual culture tests, it produced a scarlet pigmentation only against *M. phaseolina*. Separate dual culture tests were also performed to ascertain the activity of antagonistic bacterium against prevalent soil fungi, *Fusarium oxysporum* f. sp. *cuminii* and a bioagent *T. harzianum*. However, *B. firmus* could not inhibit the growth of any of these fungi nor any scarlet pigmentation was observed during interaction. *Bacillus* sp. is particularly suited for studies on biological control due to its omnipresence in soils, high thermal tolerance, rapid growth in broth culture and ready formation of resistant spores. They are uniformly distributed throughout the soil rather than being concentrated in the plant rhizosphere. The main characteristics of *B. firmus* includes its thermal tolerance (45°C), phosphate solubilizing nature, compatibility with *T. harzianum*, increased nodulation, plant growth promotion and potential to colonize roots.

Development of bio-formulated products

The bio-formulated products are *Trichoderma harzianum*, *Aspergillus versicolor*, and *Bacillus firmus*.

To introduce an antagonist in the ecosystem and to help it survive, proliferate, become active and establish itself a new environment, proper formulations need to be evolved. Once a bio-control agent is selected on the basis of its potentiality and production of biomass then suitable formulation development becomes the major concern. Formulation of antagonist biomass in suitable form is another step for the success of biological

control. The formulation must be easy to handle and deliver at the site of action.

Trichoderma harzianum: After a series of experiments, efforts were made to develop bioformulated products by utilizing readily available waste materials for preparing compost, inert carrier and farmyard manure with suitable moisture where this bioagent could survive for 120 days. The bioformulated product was coined as Maru Sena 1. Till now thousands of packets of this product were made available to the farmers.

Aspergillus versicolor: Bioformulation of *A. versicolor* in a mixture of sterilized neem compost and talc was prepared, which was able to maintain viable propagules of *A. versicolor* for more than 120 days. The bioformulated product was distributed to selected farmers, as Maru Sena 2. Information gathered from *Fusarium* sp. infested fields of farmers where 10 to 15% reduction in wilt incidence was recorded, indicated that bio-formulated product could maintain aggressiveness of the bio-control agent. Use of *A. versicolor* was an additional advantage in hot arid region due to its thermal resistance (up to 65°C) and ability to survive under low soil moisture conditions. Subsequently, demonstrations were taken at farmers' field in different seasons where pronounced reduction in wilt incidence in cumin was recorded. This native biocontrol agent was successful in establishing itself in soil as recorded by calculating colony forming units. It released many types of metabolites helpful in growth promotion of plant by showing antagonistic action against soil-borne pathogens. In spite of all these positive properties there were some ailing effects of this strain. It produces mycotoxins, which were harmful to both plants and human beings. It causes aspergillosis in human being, a lung infection, and in plants it is responsible for causing mycotoxin contamination, which reduces food value. Therefore, proper research data needs to be generated before it can be recommended for crop protection in the region.

Bacillus firmus: Many locally available on-farm wastes were evaluated to select food substrates that can improve the shelf-life of *B. firmus* for a reasonable period. Our efforts successfully culminated in identifying a suitable food substrate, which was combined with carrier to retain adequate moisture in the bioformulated product so that bacterium could survive for 180 days. The product designated as Maru Sena 3 was made available for distribution to the farmers (Fig. 1). In the last 5 years, hundreds of hectare land was sown with bacterium coated seeds. Farmers were getting positive response in checking incidence of dry root rot in rainfed crops.

Consortium of bio-control agents

Incidentally, *B. firmus* sp. operates through antibiosis, while *Trichoderma harzianum* kills the mycelium of the pathogen mainly through hyper parasitism. Both the bioagents were compatible in nature. Strategies for developing mixtures of bio-control agents were envisioned including combining effective organisms against single or more pathogens. It was, therefore, thought worthwhile to develop a consortium of both the native bio-control agents in the form of a single bio-formulated product to improve pathogen control by way of operating different mechanisms of antagonism. Utilizing readily available cheap food substrates and inert materials, a new bioformulated product encompassing both the bio-agents was developed, wherein these organisms could survive for 120 days. The product designated as Mishrit Maru Sena, can be stored well at room temperature without losing viability of propagules/ spores of both the bioagents under wide range of electrical conductivity.

Field demonstrations

After developing bio-formulated products, their efficacy was demonstrated at farmers' field to disseminate this cost effective and easy management strategy in the region. Field demonstrations were carried out at adopted villages of the Institute's Krishi Vigyan Kendra

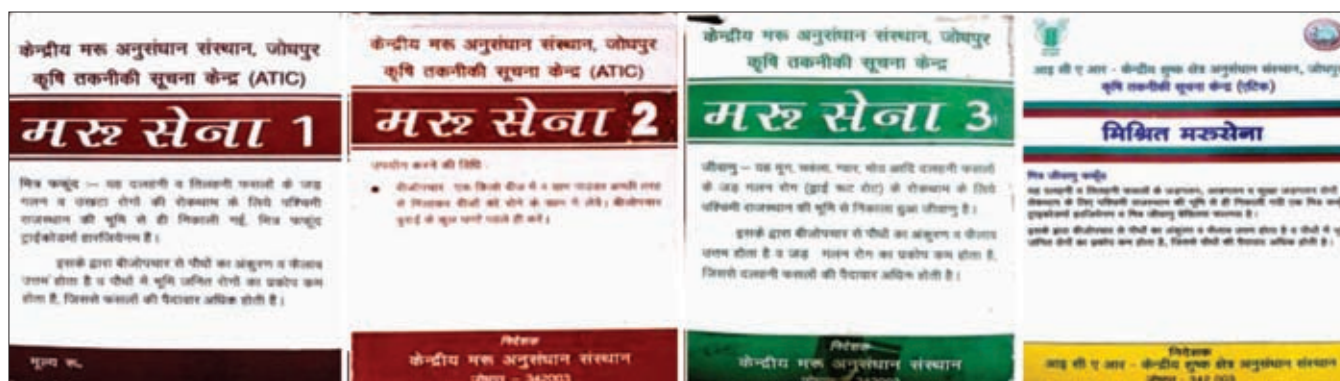


Fig. 1. Bioformulated products of bio-agents

(KVK). Effectiveness of seed coating with Maru Sena 1 on incidence of dry root rot and seed yield of *guar*, moth bean and sesame, and on incidence of wilt in cumin were demonstrated at 290 farmers' fields from 2015 to 2017. *Guar*, moth bean, sesame and cumin seeds were coated with bio-formulated product (4 g/kg seeds) and in case of *B. firmus* mixer of bio-formulated product were dissolved in jaggery suspension before coating the legume seeds. At each demonstration, 800 m² areas each were planted with bioagent coated and uncoated seeds within 24 to 48 hr. of coating. Plant mortality due to dry root rot and incidence of wilt were recorded 55 to 60 days after sowing in 5 randomly selected plots (4 × 4 m²) at each site, while seed yield was recorded after harvesting. Seed yield increased by 13.3 to 23.5% just by seed treatment in all the legumes and oilseed crops in these demonstrations. The effect of seed coating was more conspicuous on local cultivars than the improved short duration genotypes. Increase in seed yield in the plots planted with *B. firmus* coated seeds ranged from 14.8 to 17.5 and 14.5% in *guar* and moth bean, respectively (Table 1). In general, better germination and early flowering was observed in treated plots. This indicated that increase in seed yield was not only due to reduced disease incidence but was also due to cumulative effect of better germination, plant growth and nodulation. Seed treatment with bio-agent resulted in considerable reduction in incidence of wilt on cumin crop at demonstrations conducted by KVK, Pali and Jodhpur during winter of 2015 -2016 resulting in 14.5 to 25.8% yield

promotion (Table 1).

Effectiveness of seed coating with bio-formulated product of consortium of *T. harzianum* + *B. firmus* (Fig. 1) on incidence of dry root rot and seed yield of *guar* demonstrated at farmers' field recorded reduced disease incidence (7.6%) than control (12.2%) resulting in 22.9% increase in seed yield on the basis of pooled average (Table 1). This product also improved root colonization of *B. firmus* providing long duration of protection to host by pathogen. Its application as seed coating along with incorporation of small dose of compost prepared from radish residues further improved root colonization.

The research efforts culminated in identifying suitable food substrates, which were combined with carrier to retain moisture in the bioformulated product so that bioagents could survive for 120 days. Demonstrations of this consortium at farmers' field, an essential component of any technology, gave encouraging results by way of reducing disease incidence and increasing yield. This is an eco-friendly and easy to apply approach for managing soil-borne plant pathogens. The expected benefit will depend on severity of the disease in a particular field and season. Thus B:C ratio may vary from 1:25 to 1:50 for crop-to-crop and season-to-season, because dry root rot occurs in severe form when crops experience moisture

Table 1. Effectiveness of Maru Sena 1, Maru Sena 3 and Mishrit Maru Sena on dry root rot and wilt incidence and seed yield of arid legumes, oil seed and cumin

Years	Crop	No. of Demonstrations*		Disease incidence** (%)		Yield** (kg/ha)		Increase in yield
				Treated	Control	Treated	Control	
Maru Sena 1								
2015	Guar	24	7.7	11.3	368	291	13.3	
	Cumin	25	13.8	20.9	686	597	15.8	
	Moth bean	4	7.2	9.5	640	550	14.9	
	Moong bean	5	6.4	10.5	870	780	11.1	
	Sesame	5	8.4	11.4	640	550	16.3	
2016	Guar	67	6.9	11.9	432	369	13	
	Moth bean	19	7.3	11.8	450	400	12.2	
	Moong bean	20	7.7	15.4	517	437	15.8	
	Cowpea	4	11.3	11.8	1380	1130	22.1	
	Sesame	5	9.7	20.5	650	530	22.6	
Maru Sena 3								
2007	Guar	22	6.81	10.8	331	282	17.5	
2008	Guar	33	10.5	16.5	352	306	14.8	
	Moth bean	6	20.4	50.2	476	416	14.5	
Mishrit Maru Sena								
2015-16	Guar	10	7.6	12.2	750	610	22.9	
2016-17	Guar	40	6.7	15.4	720	590	22	

*In different villages of Jodhpur and Pali districts,

**Pooled average of demonstrations for all the crops

stress of short and long durations. Use of biocontrol agent is only one component of integrated disease management. To obtain better results it should be integrated with other recommended management strategies.

As a part of awareness programme, four on-farm farmers trainings and group discussions on seed treatment of legumes and cumin with Maru Sena, as one of the management strategy in the integrated disease management of soil-borne plant pathogens, were organized in the adopted villages of the Institute and Krishi Vigyan Kendras in participatory and interactive mode. Growers of this region will be benefitted by adopting this easy and cost effective technology to reduce chronic soil-borne diseases in economically valuable crops to get a better harvest.

The process of developing bio-formulated products of these bio-pesticides (Maru Sena-3 and Mishrit Maru Sena) were filed for patenting so that the product can be put to commercialization for wider adaptability among growers. (Published in *Indian Patent Site* in November 2014 and January 2015 respectively).

Khejri rejuvenation

In recent years, Jodhpur, Pali, Nagaur and Sikar are experiencing acute problem of *khejri* (*Prosopis cineraria*) mortality, due to *Ganoderma lucidum*, root rot causing soil-borne plant pathogen. *Khejri* mortality is fast spreading to other adjoining districts also. The protection technique involved digging a pit around the trunk during rainy season until the lower portion of the branching roots become visible and applying phorate granules (20 g) to protect the tree from the attack of *Acanthophorus* sp. at the base of the pit. After layering a cover of soil over this application, *Trichoderma*



Fig. 2. Rejuvenation of Khejri (A) Before, and (B) after six months of treatment

harzianum amended with *Prosopis juliflora* compost and farmyard manure (5 to 7 kg) is applied. Irrigation is also given to spread *Trichoderma* in the whole pit and to provide additional moisture. Further one layer of another bio-control agent *Aspergillus terreus* amended with onion residues (2 kg) is applied to protect the tree from the attack of *Ganoderma*, and irrigation is given to spread the biocontrol agents in the whole pit and to provide additional moisture to establish the bioagent beneath the tree collar region. The effectiveness of biocontrol agents, compost and residues was established. The treatment is repeated twice at six monthly intervals. In September 2016, above treatment was applied in five *khejri* trees at Rajorki Dhani, a village of Luni tehsil in Jodhpur district, and after 6 months remarkable rejuvenation growth was observed in these treated *khejri* trees which was measured as canopy dia and light intensity reading using Lux meter (Fig. 2).

SUMMARY

Our endeavor to prepare and demonstrate use of bioformulated

products of bioagents resulted in considerable success to disseminate a non-chemical, cost effective and easy application strategy for managing soil-borne plant pathogens of crops grown under rainfed and irrigated conditions in arid region. Resource deficient growers are not able to adopt technologies where lot of water and expensive external inputs are required. Therefore, use this low input sustainable agriculture technology. Use of bioagents does not require any expensive resources; more so, these products are readily available and can be used by farmers just after an initial on-farm training. These products are made available for distribution to the farmers through (ATIC) of the Institute. In the last 10 years, more than 1,000 ha of land was sown with bioagents coated seeds in the region. The process of developing bioformulated product of these bio-pesticides was published by Indian patent site to put these products for commercialization for wider adaptability among growers.

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