Integrated crop disease management in arid Rajasthan: a synthesis of indigenous knowledge with biocontrol

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In view of awareness toward nature-friendly management of plant diseases, the need of integration and use of indigenous knowledge (IK) with modern biological control measures has been emphasized. In view of new insights being generated it is accentuated to reassess the system of sustainable plant disease management. One farmer-inspired indigenous practice of using raw cow milk as seed treatment has been experimentally validated integrating with Trichoderma spp. (the farmer-friendly fungus and biocontrol agent) at farmers' fields and at C. R. Farm of the Central Arid Zone Research Institute, Jodhpur. The present article, besides documenting the success stories of validating biocontrol agents to manage plant diseases in arid Rajasthan, attempts to revive interest in IK and biocontrol emphasizing new research needs to reassess the system of sustainable plant disease management.

Keywords: Arid zone crops, biocontrol, disease management, indigenous knowledge, Trichoderma spp.

IN view of the fast changing agricultural scenario, a drift has resulted from sustenance to commercial farming. To maximize the yield, farmers are using high-yielding varieties and hybrids with higher inputs of chemical fertilizers and pesticides to a large extent. The newly released hybrids, indiscriminate and injudicious use of fertilizers and pesticides have resulted in susceptibility to various diseases and insect pests. Besides this, a number of other problems such as soil, water and air pollution, residual toxicity in fruit and vegetables, resistance to insects and pathogens, mortality of parasites, predators and pollinators, and resurgence with outbreaks of secondary pests have also cropped up.

With increasing environmental awareness, the focus has now shifted towards the search for viable alternatives of disease control methods. Amidst a web of high throughput technologies, low external input sustainable agricultural (LEISA) technologies are in great demand. Sustainability is a new challenge for modern agriculture. Answering this challenge will take the form of a dialectic between our understanding of available practices and our expanding knowledge of ecological relationship in agro-ecosystems.

Indigenous knowledge (IK) is the systematic body of knowledge acquired by local people through the accumulation of experiences, informal experiments and intimate

understanding of the environment in a given culture. These practices have sustained the farmers from the ancient times. In other words, IK is tuned to the needs of local people, and the quality and quantity of available resources, along with a natural system of preserving ecological balance. The efficiency of indigenous practices lies in the capacity to adapt to changing circumstances and recycling of natural resources. IK is a product of experience followed by informal experimentation. In scientific colloquium, IK is analogous to technology generation as conceived in on-farm trials. However, formal experiments are required for the function of technology validation. Experimental validation involves an assessment of the significance of IK and relevance in solving problems, reliability, i.e. not being an accidental occurrence, functionality (how well it work), effectiveness and transferability. In other words, the application of a scientific validation is based on scientific criteria that purportedly separate the useful from the useless, objective from subjective, and indigenous 'science' from indigenous 'beliefs'. Despite a plethora of evidences on field efficacy of IK, a great resurgence of interest in biological control and inclusion of practices in integrated disease management (IDM), the relative volume of the literature on technology validation, especially through understanding the mechanism of action is meagre.

The present article is thus based on the experimental verification of a farmer-inspired indigenous technology of using raw cow milk (RCM) with integration of *Tricho-derma* spp. to manage some of the most economically

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important diseases of arid zone crops and trees. The validation experiments were conducted during the last one and a half decades at the Central Research Farm of Central Arid Zone Research Institute (CAZRI), Jodhpur along with on-farm experiments at various farmers' fields of Osian tehsil, Jodhpur district.

Validating people's knowledge: some case studies

Practically sound and encouraging results were recorded when validation of the use of milk and *Trichoderma* spp. against major fungal and viral diseases of arid zone crops were made at CAZRI. These results are discussed here as case studies of using IK practice of RCM with species of farmer-friendly fungi *Trichoderma viride* and *Gliocladium* (syn. *Trichoderma*) virens.

Raw cow milk and G. virens against downy mildew of pearl millet

Downy mildew (DM) of pearl millet is the most important disease caused by *Sclerospora graminicola* (Sacc.) Shroet. occurring in all the millet-cultivating tracts of India (Figure 1). Pre-sowing treatment of seed with systemic fungicides is commonly used to manage the disease¹. However, the lack of durable resistance, existence of pathogenic variability and concerns about fungicide resistance have limited the potential of such strategies for managing the disease. In view of poor economic value of the crop use of biological control as an alternative, environment-friendly means for the management of the disease has become important.

Studies were undertaken to manage DM in rainfed crop of pearl millet employing RCM with *G. virens* as seed and soil treatments. A field experiment was conducted during the rainy season in a DM sick plot at CAZRI. 'Nokha-local', a DM-susceptible pearl millet cultivar was

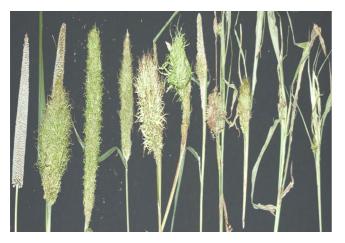


Figure 1. Green ear heads showing types of malformations with a healthy ear head (extreme left).

used. The experiment was conducted with five treatments in a randomized block design (RBD) with three replications: (i) Seed treatment with RCM (50%, diluted with water) for 18 h at room temperature $(30 \pm 2^{\circ}C)$; (ii) seed treatment with G. virens (6 g kg⁻¹ seed); (iii) soil treatment with G. virens (10 g m^2) ; (iv) seed treatment with RCM + G. virens and soil treatment with G. virens and (v) control (no soil and seed treatment). Each plot measured $3 \text{ m} \times 2 \text{ m}$, with four rows and each row had 20 plants. The crop was fertilized with diammonium phosphate (40 kg ha^{-1}) as basal dose. No insecticides or herbicides were applied. DM incidence was recorded twice, 30 days after sowing (DAS) and at soft dough stage (i.e. 60 DAS). Fresh weight/dry weight ratio of 50 plants and 1000-grain mass were analysed for all the treatments.

In terms of disease incidence and protection over control, seed treatment with RCM seems to be similar to soil treatment with G. virens. However, a combination of all the three treatments (i.e. seed treatment with RCM and G. virens and soil treatment with G. virens) did not show significant difference from seed treatment with G. virens for disease incidence and control². Results indicated some additive effect of RCM probably through induced resistance. Proline and potassium phosphate in RCM are known to boost the immune system in plants. Irrespective of the treatments, almost all yield attributes showed little change. Plant height was maximum in the treatment where G. virens was added to the soil, followed by seed treatment with G. virens. Highest root length was recorded in seed treatments with RCM and G. virens, whereas about 7.3% increase in plant height was recorded in soil treatment with G. virens. The fresh weight/dry weight ratio of 50 plants decreased by 12% in the combination of all three treatments followed by soil and seed treatments with G. virens. This indicated that biocontrol treated plants curtailed the process of rapid necrosis and drying. Maximum 1000-grain mass was observed in RCM seed treatment (21.3 g) followed by G. virens applied to soil (19.3 g) and G. virens seed treatment (18.6 g). The combination of all three treatments had lowest (17.0 g) 1000grain mass².

These treatments provided encouraging results with 72.9% protection over control^{2,3}. This fact indicated that these agents may facilitate defence response in pearl millet against DM disease. The effects of RCM and *G. virens* were further examined on the possible induction of defence-related metabolites and enzymes for their ability to induce DM resistance. Pearl millet seed priming and analysing changes in a number of key plant biochemical parameters for biocontrol treated and untreated (control) pearl millet plants to correlate those changes with the resistance induced in the treated plants were made. Besides this, the activity of photosynthetic pigments was also observed. Results revealed that concentrations of all pigments were reduced in untreated (control) leaves when

compared to the leaves of treated plants. The chlorophyll *a*, *b* and total chlorophyll in treated plants were observed higher by 22%, 59% and 31% respectively, in healthy leaves of treated plants. Results further showed that in the diseased leaves of treated plants the level of chlorophyll *a*, *b* and carotenoids was much higher with 76% increase in chlorophyll *a*; 141% in chlorophyll *b*, 90% in total chlorophyll and 106% in carotenoids in comparison to the healthy and diseased leaves of control plants⁴.

Phenolics are substances that are involved in plantpathogen interactions. The total phenolic content showed increase in healthy (2%) and diseased leaves (10%) of treated plants when compared with those of control plants. Results showed that free amino acids reduced by around 11% in healthy leaves and about 18% in the diseased leaves of treated plants. Since little information is available in the literature about the role of proline in inducing resistance in plants at the biochemical level⁵, evaluation of endogenous proline content in the leaves of treated and control plants revealed that free proline content was reduced by 47% in the healthy leaves and 43% in diseased leaves of treated plants in comparison to the corresponding healthy and diseased leaves of control plants. This suggests that the leaf tissues in control plants are under senescence.

Results revealed that the enzyme levels were considerably higher in treated plants than in water-treated control plants. High activity of polyphenol oxidase (PPO) was recorded in both healthy (184.2%) and diseased (27.72%) leaves of RCM and G. virens (biocontrol agents; BCAs) treated plants when compared to the corresponding control plants. However, low PPO activity (58.13%) was recorded in healthy leaves when compared to the diseased ones in treated plants. The same was also found true in case of control plants. Peroxidase (POX) activity was also seen to increase (28.8%) in healthy and diseased (27.7%) leaves of BCA treated plants. Interestingly, the catalase (CA) activity was higher in healthy and diseased leaves of the BCA treated plants by 45.7% and 47.5% respectively. However, soluble proteins were seen to decrease in the treated plants in comparison to the control ones.

In this study, an attempt has been made to analyse changes in a number of key plant biochemical parameters. The study corroborates those previous findings and found that diseased control plants had a significant increase in soluble sugar concentration when compared with the treated ones. In the present study, we report the involvement of PPO, POX and catalase during the pearl millet and DM disease interaction. Effective DM management requires a definite reduction in primary inoculum from seed and soil. On this count, *G. virens* appears to have grown readily along with the developing root system of the treated plant and protects the roots from initial infection.

There is a long tradition of indigenous innovations involving prophylactic use of milk and its derivative for

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controlling diseases in plants as well as animals in India. As low economic value pearl millet crop is largely grown by resource-poor farmers, seed treatment with biocontrol agents is a more viable and less expensive option than spraying of fungicides to control DM. Unlike chemical fungicides having high risk of developing resistance in the pathogen, biocontrol agents are free from such threats. As a treatment option RCM and *G. virens* are promising for pearl millet DM management. Apart from their action against DM, these treatments are good plant growth promoters, which is an added benefit for advantageous cultivation of pearl millet.

Raw cow milk and Trichoderma-induced protection against diseases of chilli

Against leaf curl disease: In order to assess efficacy of a bio-management strategy for leaf curl disease (LCD) of chilli, extensive 'on-farm' experiments were conducted in farmers' fields of Mathania, Narwa and Manai villages of Jodhpur district in western Rajasthan. Chilli seeds were treated with RCM for 24 h in 1 : 1 ratio (i.e. RCM diluted to 50% by adding water) at the room temperature ($30 \pm$ 20°C) and *T. viride* (6 g kg⁻¹ seed) and *T. viride* (10 g m⁻²) in nursery soil followed by dipping of nursery-raised saplings in RCM (15%) for 20 min before transplantation. After 20 days of transplanting, the plants were sprayed with RCM (15%) four times at 15 days interval. The farmers' practice (FP) was treated as control.

Treatment of biocontrol agents was found superior over FP in all the trials providing about 17–65% (mean 48.4%) protection over FP (Figures 2 and 3). Yield attributes like plant height, root length, number of branches per plant, number of fruits per plant, fruit size, fruit weight and fruit yield per plot showed an increase when compared to FP. Besides reduced incidence of LCD and yield attributes, the net monitory return was more (Rs 8849.47 ha¹) in the treatment of bio-agents in comparison to FP with benefit : cost (B: C) ratio of 1.68 : 1.31 in the treatment and FP respectively⁶.



Figure 2. Raw cow milk and *Trichoderma* treated (left) and control plant (right) in pot experiments under net-house condition.

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The protection is based on the stimulation of defence mechanisms by metabolic changes along with increase in defence-related enzymes such as polyphenol oxidase and peroxidase that enabled the plants to defend themselves more efficiently against LCD virus.

Against die-back disease: Die-back disease is caused by a fungus Colletotrichum capsici (Syd) Butler and Bisby, which is also responsible for causing anthracnose and ripe fruit rot. The disease appears during October to January affecting plants within a month of transplanting and causes necrosis of the tender twigs from the tip backwards. Disease is characterized by the appearance of water-soaked, brown necrotic lesions in 1-5 cm long patches on the stem above the collar regions. In advanced stage of infection the lesions turn greyish-white to straw colour and the entire plant dries up. The damage potential of disease is enormous, affecting the crop by reducing the height of affected plants, root length and number of branches on the main stem. The causal fungus is seedborne in nature. At the time of maturity, the fungus invades the fruits and enters into the seeds. It also infects the seedlings growing from infected seeds and continues to attack leaves and stems during the growing season. The fungus survives in the field, in plant debris and through seeds collected from diseased fruits. Secondary spread of pathogen takes place through wind-borne conidia. As the disease is seed, soil and air borne in nature, it is difficult to manage in field conditions. There is no chilli line reported to be immune to this disease. In view of this fact, a two-year 'on-farm' experiment was conducted in the farmers' fields at the real 'hot spots' of the disease in Mathania and Tinwri villages of Jodhpur district (Box 1). Chilli seeds were treated with RCM for 24 h in 1:1 ratio (i.e. RCM diluted to 50% by adding water) at the room temperature (30 \pm 20°C) and T. viride (6 g kg⁻¹ seed) and T. viride (10 g m^2) in nursery soil. FP was treated as control.

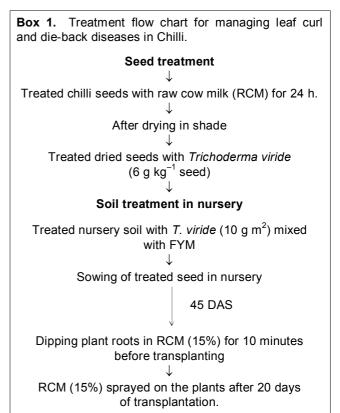
The biocontrol agents (RCM and *T. viride*) could manage the die-back disease from 20% to 77.3% (mean 39.2%)



Figure 3. Efficacy of raw cow milk and *Trichoderma viride* seed treatment with *T. viride* application in soil (left) and farmers' practice (right) at a farmer's field.

protection over control). Yield attributes like plant height, root length, number of branches per plant, number of fruits per plant, fruit size, fruit weight and fruit yield per plot showed an increase when compared to FP.

Biological control of cumin wilt disease: Jeera, i.e. cumin (Cuminum cyminum L.) is predominantly a rabi season crop. India is the world's largest producer and consumer of cumin. Most of the demand for cumin seeds is from the food and food-processing industries; the seeds are used as a condiment or spice in curries, pickles and in cooking besides their use in some medicines. The crop is mainly cultivated on 264,000 ha with a production of 108.7000 tonnes in Rajasthan, Gujarat, Madhya Pradesh, Haryana, Punjab, Uttar Pradesh and Bihar⁷. Among these, Rajasthan contributes maximum area as well as production. Major cumin cultivation areas in Rajasthan are Jalore, Barmer, Nagaur, Jodhpur, Pali, Ajmer and Tonk districts⁸. Rajasthan is a leading producer of cumin accounting for 70% of the all India output. The districts of Jalore, Jodhpur, Barmer, Nagaur and Pali account for 90% of the total production in Rajasthan. However, the average productivity of the crop in the arid western Rajasthan is very low (392 kg ha⁻¹). The susceptibility of popular cultivars to various diseases is the main reason for low yield of cumin in the area. The foremost disease responsible for low yields and poor quality in cumin is wilt caused by Fusarium oxysporum f. sp. cumini.



Wilt disease: Attack of wilt is severe in relatively younger plants. The disease first affects the root of a plant, and the affected plant dries up. Infected plants show peculiar symptoms of dropping of tips and leaves, leading to mortality of the entire plant. Results in yield losses have been reported from 35% to 65% in some districts of Rajasthan^{9,10}.

Since the pathogen is soil and seed borne, the spores survive in the soil for years together in the form of resting spores known as chlamydospores, even in absence of cumin crop. Schemes to eradicate the pathogen are limited by the ability of the fungi to survive in the soil for long periods, with or without a host plant, and the colonization of the vascular tissues within a plant. To promote export potential of the crop fetching it good price in the international market, it is necessary to reduce indiscriminate use of pesticides, which leads to serious environmental pollution, resistance in pathogens and human health hazards. The present scenario thus necessitated the development of alternative ecofriendly management strategies for cumin wilt disease.

'On-farm' studies were conducted in the Organic Agriculture Farm at the Central Research Farm area of CAZRI, for managing cumin wilt using environment-friendly technology as follows:

- 1. Seed treatment with RCM (9% dilution with water) for 20 h.
- 2. Seed treatment with RCM (9% dilution with water) for 20 h and *G. virens* (6 g kg⁻¹ seed).
- 3. Seed treatment with G. virens (6 g kg⁻¹ seed).
- 4. Seed treatment of sterile distilled water (control).

All the treatments with biocontrol agents showed considerably less incidence of wilt when compared to the control plants. Results revealed that the highest (83.5%) protection of disease over control was recorded in seed treated with *G. virens* with 3.3% disease incidence followed by seed treatment of RCM and *G. virens*, and seed treatment only with RCM. Maximum seed germination (73%) was observed in seed treated with *G. virens*. However, the high seed yield of 8.8 kg ha⁻¹ was obtained in RCM and *G. virens*-treated plants. Rest of the treatments exhibited almost the same seed yield (Table 1).

The present study has demonstrated that under field conditions, seed treatment with RCM and *G. virens* reduced wilt incidence and offered considerable disease protection. These treatments, apart from their action against cumin wilt disease, are good plant growth promoters, which is profitable for cumin cultivation.

Rejuvenation of Ganoderma affected khejri trees through biocontrol agents

Khejri (*Prosopis cineraria* (L.) Druce) is a drought-hardy and multipurpose tree of semi-arid areas. This is a highly

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valued tree of desert ecosystem as renewable source of energy and biomass. The tree is used as food (vegetable, dry fruit), feed and is also a rich source of fuel. It enriches the soil and improves the growth of various aridzone crops. It is available in abundance in protected agriculture lands because of its eco-friendly nature. The tree is therefore termed as the backbone of rural economy and has become an integral part of traditional agro-forestry system. It can grow on a variety of soils, but preferably on deep sandy loam with availability of moisture. The tree is so hardy that it can survive even under dry (less than 100 mm annual rainfall) and harsh climatic conditions (temperature as high as 48°C). Recently, heavy mortality of these trees has been reported from various parts of Rajasthan and has caused serious concern. Khejri is the 'State tree of Rajasthan' and its sudden death has caused anxiety among the farmers, environmentalists and scientists. The mortality of adult trees was found as high as (5%) in Nagaur, Jhunjhunu, Jodhpur, Churu, Sikar and Jaipur districts of Rajasthan. Pathological investigations revealed that white rot fungus, Ganoderma lucidum impaired the nutrient and water transport system of the fully grown trees. Moreover, Ganoderma fungus is found to grow and parasitize the basal portion (roots and stems) of trees. Therefore, the disease is also known as basal stem rot. It is a major limiting factor for survival of the age-old khejri plantation. Several workers reported different management practices to contain the mortality of khejri, but the results are not consistent. Now, fungal BCAs have been proved to be a potent biocontrol measure against soil-borne plant pathogenic fungi. A number of technical, economical and environmental factors stimulate the use of biocontrol agents for the control of Ganoderma pathogens. Trichoderma species are reported all over the world for their beneficial uses, not only in disease control, but also in improvement of plant health. Recently, BCAs have emerged as a modern strategy to manage plant diseases.

In view of this development, efforts were made to develop a suitable management practice using BCAs to hold the dreaded disease. To achieve success, native strains of *Trichoderma* and *Gliocladium* were used for the recovery of partial to severely affected trees. Most of affected trees recovered from drying stage and started showing new growth (production of new flushes of green leaves) after BCA treatment. The factors responsible for regeneration of drying trees were basically the use of potential BCAs, multiplication of BCAs on active medium and maintenance of proper moisture for keeping viability of BCAs during field application.

Rejuvenation of gigantic sacred tree – $R\bar{a}m$ Khejda: The sudden drying up of 256-year-old and religiously important Khejri tree was seen in Kherapa village (Jodhpur–Nagaur NH65), Jodhpur district. The tree was healthy until May 2003, and dried up suddenly and became

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Treatment	Disease incidence ^a (%)	Protection over control (%)	Seed germination (%) ^b	Yield (kg ha ⁻¹) ^c
Seed treatment with raw cow milk (9% dilution with water) for 20 h	5.4	73.0	55	7.6
Seed treatment with raw cow milk (9% dilution with water) for 20 h and <i>Gliocladium virens</i> (6 g kg ⁻¹ seed)	6.7	78.5	70	8.8
Seed treatment with G. virens (6 g kg ^{-1} seed)	3.3	83.5	73	7.8
Seed treatment of sterile distilled water (control)	20.0	-	65	5.1
CD at 5%	1.3	10.7	27	0.7
CV (%)	85.49	11.47	14.23	21.47

Table 1. Effect of biocontrol agents on cumin wilt incidence, protection over control and seed yield

^aRecorded at 85 days after sowing. ^bSeed germination recorded at room temperature 22 ± 3 °C. ^cPlot size 3×4 m.



Figure 4. Rejuvenation of khejri (*Prosopis cineraria*): *a*, Main gate of Khedapa Ramdwara; *b*, Dead and newly grown tree; *c*, Same tree showing good growth; *d*, Inscription of the name of the newly developed tree on a stone slab as 'Shri Ram Khejda'; *e*, Ramdwara Guru Gaddee.

leafless. On the basis of encouraging results obtained earlier with BCAs isolated from native soil and diverse habitats (such as sick plant, decaying wood and fruiting bodies of G. lucidum), the affected tree was treated with potential strains of BCA. In this case, sick soil and affected tree parts along with Ganoderma fungus were removed. A circular ring around the periphery of the trunk (4' deep and 2.5' wide) was prepared. Four holes (2-4 cm wide and 5-10 cm long) were drilled in woody roots and trunk with electric driller and then inoculated with potential strains of T. pseudokoningii and G. virens (GTP-7 and GGV-3) in July 2003. The potential strains of Trichoderma and Gliocladium were mixed with jaggery to pour the slurry inside the drilled holes of the trunk and roots. Biocontrol fungi were also mixed with FYM to treat the affected soil in ring basin. The tree started rejuvenating in December 2003 by sprouting new growth from the root zone which was treated with biocontrol agents and other additives. Revived shoots were protected with wire-net so that they can attain a proper growth and to protect them from abiotic and biotic damages. The tree was further given follow-up treatments of BCAs (GTP-7 and GGV-3) with micronutrients in FYM

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having 16 branches.

people at large.

Information on traditional practices for managing plant diseases has been poorly documented. The Indian Council of Agricultural Research, New Delhi has published a set of documents in seven volumes during the period 2002– 2004 documenting 4879 indigenous practices in agriculture, generated through a Mission-Mode Project on collection, documentation and validation of practices. These have been published under the general title of 'Inventory of indigenous technical knowledge in agriculture'¹¹. But these volumes mostly contain validation of

around the root zone and rejuvenated sprouts for further

development into a 'young tree' (Figure 4c). The tree has

now attained a height of 11.5 ft, with 1 ft collar diameter

have an important role to play in managing plant patho-

genic fungi causing root and butt rots. Presently, the new

(recovered) tree is young with lush green foliage and true

to type. The rejuvenated tree has reinstated faith among

The above story demonstrates that these native strains

insect pests and there are only a few examples of diseases/disorders.

Biological control, which includes elicitors of host defence, microbial antagonists and natural products, offers an attractive alternative to synthetic pesticides. A large number of plant diseases have been managed using biocontrol agents. What is important now is to discover and use the natural biological control mechanisms evolved so far against the plant diseases. An increasing number of biocontrol agents are now being introduced into the market; but commercialized systems for the biological control of plant diseases are limited in number. However, some biocontrol agents have been reported to be as effective as fungicide control. In view of the awareness towards nature-friendly management of plant diseases, use of biological control measures will be a promising proposition for disease management. The fact is to be reckoned with that with genuine concern for the environment and quality food, the extent of use and support to IK and biological control is way behind the chemical technology. The reasons are many, e.g. lack of proper standardization, product formulation, industrial production and value addition in IK technologies. A new approach to research-extension linkage is needed to address these issues and fill the gaps for a sustainable and environment-friendly agriculture.

RCM is an excellent source of nutrients and offers an exceptional medium for exploration. Cow milk is known to have amino acids and potassium phosphate which help in boosting the immune systems of plants through induced resistance¹². Reports are available on the effectiveness of milk as abiotic inducer of resistance in susceptible plants^{13–16}.

The main reason why grass root innovations were being ignored was because peer pressure often forced scientists to focus on high-impact research with wide visibility. The situation is changing now with a horizontal emphasis on ecological and quality concerns. Recent patenting of a milk-based product active against a number of fungal diseases in general and mildews in particular from the Horticulture and Food Research Institute of New Zealand Limited¹⁷ is, in fact, a matter of recognition to IK and farmers' wisdom.

Now it is strongly advocated to strengthen such systems through village-based initiatives and actively involve local farmers for successful sustainable agriculture and rural development programmes. In fact, we are learning how to best utilize these traditionally applied natural tools for meeting the next challenges of agriculture. It requires alternative technologies in order to feed the burgeoning population while reducing the input of chemical pesticides in our food chain and the environment.

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