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Review Article

Experimental Validation of Indigenous Knowledge for Managing Crop Diseases in Arid Rajasthan

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Abstract

In view of awareness towards 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 CAZRI, Jodhpur. The present paper, besides documenting the success stories of validating biocontrol agents to manage plant diseases in arid Rajasthan, attempted to revive interest in IK and biocontrol emphasizing new research needs to reassess the system of sustainable plant disease management.

Keywords : Indigenous knowledge, validation, raw cow milk, *Trichoderma* spp., arid zone crops, disease management.

Introduction

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 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 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. The 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, the formal

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experiments are required for the function of technology validation. Experimental validation involves an assessment of IK's significance and relevance in solving problems, reliability i.e. not being an accidental occurrence, functionality (how well does 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, indigenous 'science' from indigenous 'beliefs'. Despite 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 literature on technology validation, especially through understanding mechanism of action is very meager.

Present paper is thus based on the experimental verification of a farmer-inspired indigenous technology of using raw cow milk (RCM) with integration of *Trichoderma* spp. to manage some of the most economically important diseases of arid zone crops and tree. The validation experiments were conducted during last one and a half decade at the Central Research Farm of Central Arid Zone Research Institute, Jodhpur along with on-farm experiments at various farmers' fields of Osian tehsil in 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 Central Arid Zone Research Institute (CAZRI), Jodhpur. These results are discussed here as case studies of using IK practice of raw cow milk with species of farmer-friendly fungi *Trichoderma viride* and *Gliocladium* (Syn. *Trichoderma virens*).

(A) Raw Cow Milk and *Gliocladium 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. Pre-sowing treatment of seed with systemic fungicides are commonly used technologies to manage the disease[1]. However, the lack of durable resistance, existence of pathogenic variability, and concerns about fungicide resistance has 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 raw cow milk (RCM) with *Gliocladium virens* as seed and soil treatments. These treatments provided encouraging results with 72.9% protection over control[2-3]. This fact indicated that these agents may facilitate defense response in pearl millet against DM disease. The effects of RCM and *G. virens* were further examined on the possible induction of defense-related metabolites and enzymes for their ability to induce DM resistance. Pearl millet seed priming and analyzing 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 plants[4].

Phenolics are substances that are involved in plant-pathogen interactions. The total phenolic content showed increase in healthy (2%) and diseased leaves (10%) of treated plants when compared with that of healthy and diseased leaves of control plants.

Results showed that free amino acids reduced by around 11% in healthy and about 18% in the diseased leaves of treated plants. Since little information is available in literature about the role of proline in inducing resistance in plants at the biochemical level[5], evaluation of endogenous proline content in the leaves of treated and control plants revealed that free proline content were reduced by 47% in the healthy 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 levels of the enzymes 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* (BCAs) treated plants when compared to the corresponding control plants. However, the 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 increased (28.8%) in healthy and diseased (27.7%) leaves of BCAs treated plants. Interestingly, the catalase (CA) activity was higher in healthy and diseased leaves of the BCAs treated plants by 45.7 and 47.5%, respectively. However, soluble proteins were decreased in the treated plants in comparison to the control ones.

In this study, an attempt was made systematically to analyze changes in a number of key plant biochemical parameters. This study corroborates those previous findings and found that diseased control plants had a significant increase in soluble sugar concentrations when compared with the treated ones. In the present study, we report the involvement of PPO, POX and catalase during the pearl millet and downy mildew disease interaction. Effective DM management requires a definite reduction in primary inoculum from seed and soil. On this count, *Gliocladium virens* appeared 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 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 for control of 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 very 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.

(B). Raw Cow Milk and *Trichoderma* induced protection against diseases of chilli

(i). *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 raw cow milk (RCM) for 24 hours in 1:1 ratio (i.e. RCM diluted to 50% by adding water) at the room temperature (30 ± 2^0C) and *Trichoderma viride* (6g Kg^{-1} seed) and *T. viride* (10 gm^{-2}) in nursery soil followed by dipping of nursery-raised saplings in RCM (15%) for 20 minutes before transplantation. After 20-days of transplanting the plants were sprayed with RCM (15%) for four times at 15 days interval. The farmers’ practice (FP) was treated as control.

Treatment of bio-control agents was found superior over FP in all the trials providing about 17 to 65% (mean 48.4%) protection over FP. Yield attributes like plant height, root length, number of branches plant^{-1}, number of fruits plant^{-1}, fruit size, fruit weight and fruit yield plot^{-1} showed an increase when compared to FP Besides reduced incidence of LCD and yield attributes, the net monitory return was more (Rs. 8849.47 ha^{-1}) in the treatment of bio-agents in comparison to the FP with benefit: cost (B: C) ratio of 1.68: 1.31 in the treatment and FP, respectively[6].

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

(b). *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 caused 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 in colour and the entire plant dries. The damage potential of disease is enormous, affecting the crop by reducing the height of affected plants, root length and number of branches on main stem. The causal fungus is seed borne in nature. At the time of maturity, the fungus invades the fruit and enters into the seeds. It also infects the seedlings growing from infected seeds and continues to attack leaves and stem during the growing season. The fungus survives in 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 the fact a 2-year 'on-farm' experiment was conducted in farmers' fields at the real 'hot spots' of the disease in Mathania and Tinwri villages of Jodhpur district. Chilli seeds were treated with raw cow milk (RCM) for 24 hours in 1:1 ratio (i.e. RCM diluted to 50% by adding water) at the room temperature (30 ± 2^{0}C) and *Trichoderma viride* (6 g Kg^{-1} seed) and *T. viride* (10 g m^{-2}) in nursery soil. The farmers' practice (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% protection over control). Yield attributes like plant height, root length, number of branches plant^{-1}, number of fruits plant^{-1}, fruit size, fruit weight and fruit yield plot^{-1} showed an increase when compared to FP.

Treatment Flow Chart for Managing Leaf Curl and Dieback Diseases in Chilli

Seed Treatment

⇓

Treated chilli seeds with raw cow milk (RCM) for 24 hrs.

⇓

After drying in shade

⇓

Treated dried seeds with *Trichoderma viride* (6g kg^{-1} seed)

⇓

Soil Treatment in Nursery

⇓

Treated nursery soil with *Trichoderma viride* (10gm^{-2}) mixed with FYM

⇓

Sowing of treated seed in nursery

⇓

Dipping plant roots in RCM (15%) for 10 minutes before transplanting

⇓

RCM (15%) sprayed on the plants after twenty days of transplantation.

C. Biological Control of Cumin Wilt Disease

Jeera, the 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 seed 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 thousand hectares with a production of 108.7 thousand tones in the states of Rajasthan, Gujarat, Madhya Pradesh, Haryana, Punjab, Uttar Pradesh and Bihar[7]. Among these Rajasthan contribute maximum area as well as production. Major cumin cultivation areas in the state of Rajasthan are Jalore, Barmer, Nagaur, Jodhpur, Pali, Ajmer and Tonk districts[8]. Rajasthan is a leading producer of cumin accounting for 70% of all India's 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 affect root of the plant, and affected plant dries out. 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 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 eco-friendly management strategies for cumin wilt disease.

'On-farm' studies were conducted in Organic Agriculture Farm managed at Central Research Farm area of Central Arid Zone Research Institute (CAZRI), Jodhpur for managing cumin wilt using environment–friendly technology consisted of following treatments:

1. Seed treatment with raw cow milk (9% dilution with water) for 20 h;
2. Seed treatment with raw cow milk (9% dilution with water) for 20 h and *Gliocladium virens* (6g kg^{-1} seed),
3. Seed treatment with *Gliocladium virens* (6g kg^{-1} seed) and
4. Seed treatment of sterile distilled water (Control).

All the treatments consisted of biocontrol agents showed considerably less incidence of wilt when compared to the control plants. Results revealed that the highest (83.5%) protection of the 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* and RCM 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.

The present investigations clearly demonstrated that under field conditions, seed treatment with raw cow milk and farmer-friendly fungus *Gliocladium 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.

D. Rejuvenation of *Ganoderma* Affected Khejri Trees through Biocontrol Agents

Khejri (*Prsopis cineraria* (L.) Druce) is a drought hardy and multipurpose tree of semi arid areas. This is highly valued tree of desert ecosystem as renewable source of energy and biomass. The tree is used as food (vegetable, dry fruit), feed and rich source of fuel. It also enriches soil and improves the growth of various arid zone crops. It is available in abundance in protected agriculture lands because of excellent eco-friendly nature. The tree is therefore termed as a 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 rain fall) 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 worries 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 grown up trees. Moreover, *Ganoderma* fungus loves to grow and parasitize the basal portion (roots and stem) of tree. Therefore, the disease is also known as basal stem rot. The disease is major limiting factor for survival of age-old *Khejri* plantation. Several workers reported different management practices to contain the mortality of *Khejri*, but the results are not consistent. Now, fungal biocontrol agents (BCA) have been proved to be a potent method 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 its beneficial uses, not only in disease control, but also in improvement of plant health. Recently BCAs have emerged as modern strategy to manage plant diseases.

In view of this development, efforts were made to develop a suitable management practice by using BCAs to hold the dreaded disease. To achieve the 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 treatments. The factors responsible for regeneration of drying trees were basically the use of potential BCA, multiplication of BCA on active medium and maintenance of proper moisture for keeping viability of BCA during field application.

Rejuvenation of Gigantic Sacred Tree- "Râm Khejda"

A sudden drying of 256-year-old religiously important "*Khejri*" tree was observed in *Kherapa* village (Jodhpur-Nagaur-NH 65) of Jodhpur district in Rajasthan. The tree was exceedingly respectable among the devotees due to its religious and historical importance. This blessed tree was healthy until the month of May, 2003, which dried off suddenly and became leafless. On the basis of encouraging results obtained earlier with biocontrol agents isolated from native soil and diverse habitats (such as sick plant, decaying wood and fruiting bodies of *Ganoderma lucidum*), the affected tree was treated with potential strains of BCAs. In this case, sick soil and affected tree parts along with *Ganoderma* fungus was removed. A circular ring around the periphery of 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 & GGV-3) in the month of July 2003. The potential strains of *Trichoderma* and *Gliocladium* were mixed with *Jaggery* to pour the slurry in side the drilled holes of trunk and roots. Biocontrol fungi were also mixed with FYM to treat the affected soil in ring basin. The tree started rejuvenating in the month of December 2003 by sprouting new growth from the root zone which was treated with biocontrol agents + other additives. Revived shoots were protected with wire-net so that it can attain a proper growth and to protect it from abiotic and biotic damages. Tree was further given follow up treatments of biocontrol agents (GTP-7 & GGV-3) with micronutrients in FYM around the root zone of affected tree and rejuvenated sprouts for further development of "New Emergence" turn into a "Young Tree". The tree has attained a height 11.5 ft. with 1ft. collar diameter having 16 branches.

The above story demonstrates that these native strains have an important role to play in managing plant pathogenic 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 disciples' and followers.

Conclusions

Information on traditional practices for managing plant diseases has never been documented. Biological control, which includes elicitors of host defense, 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. Albeit, intensive activity is currently being geared toward the introduction of an increasing number of biocontrol agents into the market, 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 awareness toward nature-friendly management of plant diseases, use of biological control measures will be a most promising proposition for disease management. The fact is to be reckoned with that with all genuine concern for 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.

Raw Cow Milk (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 immune systems of plants through induced resistance[11]. Reports are available on the effectiveness of milk as abiotic inducer of resistance in susceptible plants[12-15].

Experts used to say that the main reason why grass root innovations were being ignored because peer pressure often forced scientists to focus on high-impact research with wide visibility. The situation is changing 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 Horticulture and Food Research Institute of New Zealand Limited[16] is, in fact a matter of recognition to the Indigenous Knowledge and farmers' wisdom.

Now it is strongly advocated to strengthen such systems through village based initiatives and actively involving local peasants are considered the keys to 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 burgeoning population while reducing the input of chemical pesticides in our food chain and the environment.

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