International Journal of Biochemistry Research & Review
1(2): 31-39, 2011

SCIENCEDOMAIN *international*
www.sciencedomain.org

Effect of Raw Cow Milk and *Gliocladium virens* in Pearl Millet against Downy Mildew Disease Caused by *Sclerospora graminicola*

Arun Kumar[1*] and P. C. Mali[1]

[1]Central Arid Zone Research Institute, Jodhpur-342003, Rajasthan, India

Research Article

Received 14th February 2011
Accepted 28th March 2011
Online Ready 9th April 2011

ABSTRACT

Pearl millet [*Pennisetum glaucum* (L.) R. Br.] downy mildew (DM) is caused by the fungus *Sclerospora graminicola* (SACC.) SCHRŐT. is the most widespread and destructive disease of pearl millet affecting yield and quality in all the millet cultivating tracts of India. Since pearl millet is a crop of low economic value grown by resource-poor farmers, conventional technological interventions are not cost feasible. Integration of indigenous knowledge with biocontrol agents appeared as a logical strategy in the present case. Studies were, therefore, undertaken to manage DM in rainfed crop of pearl millet using raw cow milk together with *Gliocladium virens*. Seed and soil treatments resulted in the lowest disease incidence. Biochemical constituents (metabolites and oxidative enzymes) were analysed to determine possible mode of action of Raw Cow Milk (RCM) and *Gliocladium virens*. A considerable increase in sugars, phenols and ortho-dihydroxy phenols (OD) in healthy and DM infected leaves of treated pearl millet plants was recorded when compared to untreated controls. A marked increase in all the photosynthetic pigments in both healthy and diseased treated plants was observed. The induction of resistance was accompanied by increased activities of defense related enzymes. It is assumed that the combination of RCM and *G. virens* is capable of stimulating different systemic responses in host plant.

Keywords: Pearl millet, downy mildew, Sclerospora graminicola, raw cow milk, Gliocladium virens, metabolites, enzymes, induced resistance;

**Corresponding author: Email: arpurster@gmail.com*

1. INTRODUCTION

Downy mildew (DM) of pearl millet is the most important disease caused by *Sclerospora graminicola* (Sacc.) Shröet. occurring in all the millet cultivating tracts of India. The disease has caused considerable yield losses, and several single-cross F_1 hybrid cultivars of pearl millet have been withdrawn during last 35 years because of high susceptibility to DM (Singh, 1995; Thakur et al., 2001). There seems to be a continuous struggle between millet breeders and rapidly evolving races or pathotypes of DM pathogen (Thakur and Rao, 1997). In a recent field survey conducted in Rajasthan, the higher DM incidence (up to 78%) was recorded on pearl millet hybrids (Rao et al., 2005). Pre-sowing treatment of seed with systemic fungicides are commonly used technologies to manage the disease (Raj et al, 2003). 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. With increasing concern regarding environmental protection and human health, the use of biological control as an alternative, environment-friendly means for the management of fungal diseases has attracted extensive attention and been considered as a potential strategy for plant disease management in recent years. An alternative procedure to protect plants against disease is to activate their own defense mechanisms by specific biotic or abiotic elicitors (Walters et al., 2005).

Biocontrol agents have emerged as a new strategy of managing plant diseases by inducing systemic resistance (ISR) in plants against diseases. The basic tenet of ISR lies in enhancing resistance in response to an extrinsic stimulus without altering the genome. The protection is based on the stimulation of defense mechanisms by metabolic changes that enable the plants to defend themselves more efficiently (Steiner and Schönbeck, 1995). A number of publications with different host-parasite systems have proven the efficacy of induced resistance (IR) against fungi, bacteria and viruses through the manipulation of the host plant's physical and biochemical properties (Verma et al., 1996, van Loon et al., 1998). The emerging paradigm of sustainability in agriculture strives to amalgamate modern technology with traditional farming wisdom. Reports are available on the effectiveness of milk as abiotic inducer of resistance in susceptible plants (Bettiol, 1999; Kumar and Verma, 2006; Kumar, 2006; Crisp et al., 2006, Ferrandino and Victoria, 2007). Studies undertaken to manage DM in rainfed crop of pearl millet using eco-friendly approach employing biocontrol agents such as raw cow milk (RCM) together with Gliocladium virens as seed and soil treatments provided encouraging results with 72.9% protection over control (Kumar et al. 2004). In spite of the intriguing capacity of RCM and Trichoderma spp. to confer protection against a gamut of diseases (Kumar and Verma, 2006; Kumar 2006), very little information is available. Therefore, our major objective was to explore the ability of RCM and *G. virens* (syn. = Trichoderma virens) to protect pearl millet against DM disease. The fact that RCM and Trichoderma successfully protect pearl millet against DM (Kumar et al, 2004) indicated that these agents might facilitate defense response in pearl millet against DM disease. It has been demonstrated that defense related enzymes have been involved in resistance against pearl millet-downy mildew interaction, and that these enzymes act as biochemical markers for induction of pearl millet downy mildew resistance (Shivakumar et al., 2003; Sudisha et al., 2011).

In the present study the effects of raw cow milk and *G. virens* were examined on the possible induction of defense-related metabolites and enzymes for their ability to induce downy mildew disease resistance by seed treatment in pearl millet together with application of *G. virens* mixed with FYM in soil.

2. MATERIALS AND METHODS

2.1 HOST AND PATHOGEN

Seeds of DM susceptible pearl millet cultivar *Nokha Local* obtained from the millet breeder of Central Arid Zone Research Institute (CAZRI), Jodhpur, India, were used throughout the study. Plants were raised in DM sick-plot maintained since 1995 in Central Research Farm of CAZRI, Jodhpur, containing heavy load of soil borne oospores of highly virulent Jodhpur pathotype of *S. graminicola* (Thakur et al., 1998). Additionally sporangial inoculum was provided by the infector-row system.

2.1.1 SEED AND SOIL TREATMENTS

Pearl millet seeds were surface-sterilized with 0.02% sodium hypochlorite for two min, followed by three washes in sterile distilled water. These seed were treated with RCM of indigenous breeds of cows (Tharparker breed) available at Krishi Vigyan Kendra (KVK) of CAZRI, Jodhpur for 18 h in 1:1 ratio (i.e., RCM diluted to 50% by adding water) at the room temperature (30 ± 2^0C) and dried under the shade. The seeds treated so were further treated with *G. virens* (6 g kg^{-1} seed) (Kumar et al., 2004). *Gliocladium virens* (10 g m^2) mixed in FYM was applied to the soils of treated plants. Disease free (control) plants of the cultivar were raised from the seeds pre-treated with sterilized distilled water. The crop was fertilized with diammonium phosphate (40 kg ha^{-1}) as basal dose before sowing and irrigated at 2-week intervals. No insecticides or herbicides were applied.

2.1.1.1 Metabolites and enzymes estimations in pearl millet cultivar

The green leaves were separated and cut into small uniform pieces. From this, representative samples of 500 mg were taken from 50-day old plants for the estimation of total and ortho-dihydroxy (OD) phenols. Total phenols and OD phenols were analyzed by adopting methods given by Bray and Thorpe (1954) and Mahadevan and Sridhar (1986). The contents of chlorophyll and carotenoids (Robbetein, 1957), total soluble carbohydrate (Yemm and Willis, 1954), free proline (Bates et al., 1973) and free amino acids (Yemm and Cocking, 1955) were estimated.

In order to ascertain the role of some antioxidant enzymes, which are important markers for resistance, in the cultivar known for their susceptibility (Nokha local) the activity of defense related enzymes was observed. The enzymes peroxidase (POX) and polyphenol oxidase (PPO) were estimated using the method suggested by Shannon et al. (1966) and Kar and Mishra (1976). The assay mixture of POX contained 2.3 mL of 0.1mL of phosphate buffer (pH 6.0) at 4^0C. The reaction mixture (0.5 mL) consisted of 0.01 M pyrogallol and 0.1mL of 0.025 M hydrogen peroxide. The addition of 0.1 mL of crude enzyme extract initiated the reaction, which was measured spectrophotometrically at 420 nm (Systronics spectrophotometer, Ahmedabad, India). The assay mixture of polyphenol oxidase (PPO) contained 1.5 mL of 0.1M phosphate buffer (pH 6.0) at 4^0C. The reaction mixture (0.5 mL) consisted of 0.01 M pyrogallol. The addition of 1.0 mL of crude enzyme extract initiated the reaction, which was measured spectrophotometrically at 420 nm at 30 seconds interval for 3 min. Enzymes catalase was estimated as reported by Mahadevan and Sridhar (1986). For catalase (CAT) the reaction mixture contained 2.7 mL of 0.1M phosphate buffer (pH 6.5) at 4^0C. The reaction mixture (0.1 mL) consisted of 0.2 M hydrogen peroxide. The addition of 0.2 mL of crude enzyme extract initiated the reaction, which was measured

spectrophotometrically at 230 nm at 15 seconds interval for 2 min. All the estimations were done in triplicate and the results reported on fresh weight basis.

Data from four replicates were analyzed for each experiment and subjected to LSD values.

3. RESULTS AND DISCUSSION

A number of chemical compounds and microorganisms (Biocontrol agents or BCAs) are reported to induce resistance against plant diseases (Walters et al., 2005). However, so far there has been no report on induction of resistance by raw cow milk and *Gliocladium* against plant diseases. In this study, an attempt was made to analyse 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.

3.1 METABOLITES

3.1.1 PHOTOSYNTHETIC PIGMENTS

The chlorophyll a, b, total chlorophyll and carotenoids contents were evaluated. The concentrations of all pigments were reduced in control leaves when compared with the leaves of treated plants. As shown in Table 1, 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 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 (Table 1).

Table 1. Effect of biocontrol agents on photosynthetic pigments of healthy & downy mildew diseased pearl millet plant leaves

Pigments (mg^{-1} dry wt.)	Treated leaves*		Untreated leaves		LSD (P≤0.01)
	Healthy	Diseased	Healthy	Diseased	
Chlorophyll a	4.45 (+21.9)**	2.57 (+76.0)	3.65	1.46	0.231
Chlorophyll b	1.72 (+59.2)	0.94 (+141.0)	1.08	1.46	0.195
Total Chlorophyll	6.18 (30.6)	3.52 (+90.2)	4.73	1.85	0.517
Carotenoids	1.36 (+47.8)	0.70 (+105.8)	0.92	0.34	0.305

***Combination of seed treatment of RCM (1:1, i.e. RCM diluted to 50% by adding water) and G. virens (0.6%) with soil application of G. virens (10 g m^{-2}).*
****Figures in the parenthesis are % changes in treatment over untreated control*

3.1.2 PHENOLS

Phenolics are substances that are involved in plant-pathogen interactions. Therefore, the contents of total soluble phenols and O-dihydroxy phenol (ODP) were determined in the soluble fraction. 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. Likewise, ODP contents exhibited 14% and 55% increase over untreated healthy and diseased leaves, respectively (Table 2).

Table 2. Effect of biocontrol agents on some metabolite in the treated and mildew diseased pearl millet plant leaves

Pigments (mg^{-1} dry wt.)	Treated leaves*		Untreated leaves		LSD (P≤0.01)
	Healthy	Diseased	Healthy	Diseased	
Total Phenol	4.73 (+1.72)**	6.36 (+10.0)	4.65	5.78	0.592
Ortho-Dihydroxy Phenol (OD)	0.58 (+13.7)	1.16 (+54.6)	0.51	0.75	0.427
	1049.8 (-49.6)	1174.9 (-42.8)	1967.7	2057.2	107.51
Free Proline	2.08 (-10.7)	2.29 (-18.2)	2.33	2.80	0.192
Free Amino acids (µg g^{-1} dry wt.)	47.10 (-32.1)	60.68 (-4.48)	69.41	63.53	3.128
Total Soluble Sugars	4.73 (+1.72)**	6.36 (+10.0)	4.65	5.78	0.592

**Combination of seed treatment of RCM (1:1, i.e. RCM diluted to 50% by adding water) and G. virens (0.6%) with soil application of G. virens (10 g m^{-2}). **Figures in the parenthesis are % changes in treatment over untreated control.*

3.1.3 FREE AMINO ACIDS AND PROLINE

Results indicated (Table 2) that free amino acids reduced by around 11% in healthy and about 18% in the diseased leaves of treated plants. Similarly, free proline contents were also considerably decreased in treated healthy (47%) and diseased (43%) leaves. Free amino acids are important indicators of the plant conditions, arising as a consequence of protein degradation in tissues under programmed cell death or senescence (Hurst and Clark, 1993). Amino acid proline has an important role in physiological and pathological stress in plants (Matysic et al., 2002*)*. Since little information is available in literature about the role of proline in inducing resistance in plants at the biochemical level (Niranjan Raj et al., 2004), 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 (Table 2). This suggests that the leaf tissues in control plants are under senescence.

3.1.4 INDUCTION OF DEFENSE-RELATED ENZYMES

Results revealed that the levels of the enzymes were considerably higher in treated plants than in water-treated control plants. High activity of 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 (Table 3). All the data presented in the tables were significant at 1% level.

Table 3. Effect of biocontrol agents on defense-related enzymes in the treated and mildew diseased pearl millet plant leaves

Enzyme (OD)* min^{-1} mg protein)	Treated leaves*		Untreated leaves		LSD (P≤0.01)
	Healthy	Diseased	Healthy	Diseased	
Polyphenol Oxidase (PPO)	0.0054	0.0129	0.0019	0.0101	0.0010
Peroxidase (POX)	6.449	8.037	4.591	5.577	0.7159
Catalase (CA)	0.1075	0.3362	0.0583	0.1762	0.0497
Soluble protein (SP)	24.307	19.82	38.089	22.967	2.6910

**Combination of seed treatment of RCM (1:1, i.e. RCM diluted to 50% by adding water) and G. virens (0.6%) with soil application of G. virens (10 g m^{-2}).*

Ecofriendly disease resistance strategies are major components of modern, sustainable agriculture. Induced resistance has emerged as a potential alternative and a complementary strategy for crop protection, which signifies the control of pathogens and pests by prior activation of plants' innate defense pathways. As milk is not a potential environmental or food contaminant; consequently it can be used in organic agriculture. In India, farmers had the tradition of using milk in managing plant diseases. Milk is known to boost immune systems in the plants and the management of several diseases caused by fungi *Sphaerotheca fuliginea* (Bettiol, 1999; Kumar and Verma, 2006), and effects of RCM seed treatment together with seed and soil treatments with *Gliocladium virens* on downy mildew disease of pearl millet are also reported (Kumar et al., 2004).

In this study, an attempt was made systematically to analyze changes in a number of key plant biochemical parameters. The key symptom of DM development is the lighter green colour. This colour change of the DM infected leaves could indicate alterations in plastid metabolism. During the disease process, a decrease in chlorophyll b levels was observed, which was followed by decreases in chlorophyll a and the carotenoids levels. This decrease probably leads to a reduction in photosynthesis, previously reported for *T. cacao* infected with *C. perniciosa* (Orchard and Hardwick, 1988). A number of possible biochemical connections for this phenomenon can be visualized. Furthermore, the reduced photosynthesis could be a negative feedback response to the augmented levels of soluble sugars in the infected tissue. In plants, sugars can work directly as gene regulation signals, attenuating the expression of several plastid-localized nuclear genes required for normal chloroplast development (Mullet et al., 1990), and their presence could reduce the need for photosynthesis and, therefore, the need of pigment synthesis (Lee and Daie, 1997; Ludewig et al., 1998). The high levels of sucrose and glucose in the infected tissues have been observed previously in other biotrophic pathosystems (Chou *et al*., 2000). 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. Moreover, the decreases in the chlorophyll concentrations during senescence has been demonstrated to be followed by increases in the concentration of soluble sugars and starch (Jongebloed et al., 2004), which are somewhat similar to characteristics found in this study. Taken together, the observed

biochemical alterations associated with the infection suggest that the plant uses unspecific mechanisms to try to eliminate the fungus, such as an increase in phenolics. However, these mechanisms seem not to be sufficient to avoid the disease suggests that a cascade of events has been triggered to cause the death of the infected organ.

Induction of resistance has been measured by using biochemical markers in the form of induction of defense related enzymes that are activated upon pathogen infection. In the present study, we report the involvement of PPO, POX and catalase during the pearl millet and downy mildew disease interaction. A number of previous studies have shown that enhanced enzyme content of POX, PPO and catalase along with decreased soluble protein is associated with induced resistance against a broad range of pathogens (Retig, 1974; Chandra et al., 2001; Ramamoorthy et al., 2002). An increase in POX, PPO and CA with decrease in soluble proteins induced by RCM and *G. virens* may be facilitating pearl millet seedlings to prevent the invasion by pathogen. Similar results were observed in studies carried out on Norway spruce (Picea abies) upon infection with Pythium dimorphum (Fossdal et al., 2001). They showed an increased peroxidase activity in infected roots.

4. CONCLUSION

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. In spite of awareness about the hazardous effects of chemical pesticides in the developed countries, recommendations to use milk in controlling diseases are few. The question arises as to whether simple innovations are to be ignored for the fact that they are uncomplicated. Since pearl millet is a crop of low economic value 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. There is a high risk of the pathogen developing resistance that is associated with the use of chemical fungicides unlike biocontrol agents. As a treatment option RCM and *G. virens* are very promising for pearl millet downy mildew disease management by seed treatment which is economical and environment-friendly. These treatments, apart from their action against pearl millet downy mildew disease, are good plant growth promoters, which is an added advantage for advantageous cultivation of pearl millet.

ACKNOWLEDGEMENT

The authors are thankful to Dr. B. L. Gajja, Principal Scientist (Agric. Economics and Statistics), CAZRI, Jodhpur, India for the statistical analyses of the data.

REFERENCES

Bettiol, W. (1999). Effectiveness of cow's milk against zucchini squash powdery mildew (*Sphaerotheca fuliginea*) in greenhouse conditions. Crop Prot., 18, 489-92.

Bray, H. G., Thorpe, W. V. (1954). Analysis of phenolic compounds of interest in metabolism. Methods Biochem. Analysis., 1, 27-52.

Bates, L.S., Waldren, R.P., Teare, I.D. (1973). Rapid determination of free proline for water stress studies. Plant Soil, 39, 205-207.

Chandra, A., Anand, A., Mandal, P.K., Saxena, P. (2001). Influence of salicylic acid on protein content and catalase activity in relation to systemic acquired resistance in cowpea against root rot. Ind. Phytopath., 54, 284-287.

Chou, H.M., Bundock, N., Rolfe, S.A., Scholes, J.D. (2000). Infection of *Arabidopsis thaliana* leaves with *Albugo candida* (white blister rust) causes a reprogramming of host metabolism. Molecular Plant Pathol., 1, 99–113.

Crisp, P., Wicks, T. J., Troup, G., Scott, E. S. (2006), Mode of action of milk and whey in the control of grapevine powdery mildew. Aust. Plant Pathol., 35, 487-493.

Ferrandino, F. J., Victoria, L. S. (2007). The effect of milk-based foliar sprays on yield components of field pumpkins with powdery mildew. Crop Prot., 26, 657-663.

Fossdal, C.G., Sharma, P., Lonnebong, A. (2001). Isolation of the first putative peroxidase cDNA from a conifer and the local and systemic accumulation of related proteins upon pathogen infection. Plant Mol. Biol., 47, 423-435.

Hurst, P. L., Clark, C. J. (1993). Post-harvest changes in ammonium, amino-acids, and enzymes of amino-acid-metabolism in Asparagus spear tips. Journal of the Science of Food and Agriculture, 63, 465–471.

Jongebloed, U., Szederkenyi, J., Hartig, K., Schobert, C., Komor, E. (2004). Sequence of morphological and physiological events during natural ageing and senescence of a castor bean leaf: sieve tube occlusion and carbohydrate back-up precede chlorophyll degradation. Physiologia Plantarum, 120, 338–346.

Kar, M., Mishra, D. (1976). Catalase, peroxidase and polyphenol oxidase activities during rice leaf senescence. Plant Physiol., 57, 315-319.

Kumar, A. (2006). On-farm management of leaf curl disease in chilli under arid farming system. J. Food, Agric. Environ., 4, 180-182

Kumar, A., Verma, S. K. (2006) Milk in the Management of Plant Diseases. In: *Bridging Gap between Ancient and Modern Technologies to Increase Agricultural Productivity* (Choudhary, S. L., Saxena, R. C., Nene, Y. L. eds.): Proc. of the National Conference held from 16-18 December 2005, Central Arid Zone Research Institute, Jodhpur-342003, Rajasthan, India, pp. 67-73.

Kumar, A. Bhansali, R.R., Mali, P.C. (2004). Raw cow's milk and *Gliocladium virens* induced protection against downy mildew of pearl millet. Int. Sorghum Millets Newslett., 45, 64-65.

Lee, J. S., Daie, J. (1997). End-product repression of genes involving carbon metabolism in photosynthetically active leaves of sugarbeet. Plant and Cell Physiology, 38, 887–894.

Ludewig, F., Sonnewald, U., Kauder, F., Heineke, D., Geiger, M., Stitt, M., Muller-Rober, B. T., Gillissen, B., Kuhn, C., Frommer, W. B. (1998). The role of transient starch in acclimation to elevated atmospheric CO_2. FEBS Lett., 429, 147–151.

Mahadevan, A., Sridhar, R. (1986). Methods in Physiological Plant Pathology. 3rd Edn. Sivakami Publication, Madras, India.

Matysik, Jörg., B. Bhalu., Mohanty, P. (2002). Molecular mechanisms of quenching of reactive oxygen species by proline under stress in plants. Current Sci., 82, 525-532

Mullet, J.E., Klein, P.G., Klein, R.R. (1990). Chlorophyll regulates accumulation of the plastid-encoded chlorophyll apoproteins CP43 and D1 by increasing apoprotein stability. Proceedings of the National Academy of Sciences, USA, 87, 4038–4042.

Orchard, J. E., Hardwick, K. (1988). Photosynthesis, carbohydrate translocation and metabolism of host and fungal tissues in cacao seedlings infected with *Crinipellis perniciosa*. *Proc.* 10th International Cocoa Research Conference.

Raj, N.S., Chaluvaraju, G., Amruthesh, K.N., Shetty, H.S. (2003). Induction of growth promotion and resistance against downy mildew on pearl millet (*Pennisetum glaucum*) by rhizobacteria. Plant Dis., 87, 380-384.

Raj, N.S., Shetty, N.P., Shetty, H.S. (2004). Proline- An inducer of resistance against pearl millet downy mildew disease caused by Sclerospora graminicola. Phytoparasitica, 32, 523-527.

Ramamoorthy, V., Raguchander, T., Samiyappan, R. (2002) Enhancing resistance of tomato and hot pepper to Pythium diseases by seed treatment with fluorescent pseudomonads. Eur. J. Plant Pathol., 108, 429-441.

Retig, N. (1974). Changes in peroxidase and polyphenoloxidase associated with natural and induced resistance of tomato to Fusarium wilt. Physiol. Plant Pathol., 4, 145-150.

Rao, V.P., Thakur, R.P., Rai, K.N., Sharma, Y.K. (2005), Downy mildew incidence on pearl millet cultivars and pathogenic variability among isolates of *Sclerospora graminicola* in Rajasthan. Int. Sorghum Millets Newslett., 46, 107-110.

Robbetein, G. (1957). Unter suchugen an strable runduzierten Blattfarbunutanten on Arabidopsis thalicana (L) Hayn Ztschr. Indulet. Abstam. U. Verbugslehase., 88, 189-252.

Shannon, L., Key, E., Lew, J. (1966). Peroxidase isoenzymes from horseradish roots. 1. Isolation and physical properties. J. Biol. Chem., 241, 2166–2175.

Shivakumar, P. D., Geetha, H. M., Shetty, H. S., (2003). Peroxidase activity and isozyme analysis of pearl millet seedlings and their implications in downy mildew disease resistance. Plant Sci., 164, 85-93.

Singh, S.D. (1995). Downy mildew of pearl millet. Plant Dis., 79, 545-550.

Steiner, U., Schönbeck, F. (1995). Induced disease resistance in monocots In: Developments in Plant Pathology: Induced Resistance to Disease in Plants (ed. R. Hammerschmidt and J. Kuc), Springer, N.Y., pp.86-110.

Sudisha, J., Kumar, A., Amruthesh, K. N., Niranjana, S. R., Shetty, H. S. (2011). Elicitation of resistance and defense related enzymes by raw cow milk and amino acids in pearl millet against downy mildew disease caused by *Sclerospora graminicola*. Crop Protection (In Press).

Thakur, R.P., Rao, V.P. (1997) Variation in virulence and aggressiveness among pathotypes of *Sclerospora graminicola* on pearl millet. Ind. Phytopathol. 50, 41-47.

Thakur, R.P., Rao, V.P., Hash, C.T. (1998). A highly virulent pathotype of *Sclerospora graminicola* from Jodhpur, Rajasthan, India. Int. Sorghum Millets Newslett., 39, 140-142.

Thakur, R.P., Rai, K.N., Rao, V.P., Rao, A.S. (2001), Genetic resistance of pearl millet male-sterile lines to diverse Indian pathotypes of *Sclerospora graminicola*. Plant Dis. 85, 621-626.

Van Loon, L.C., Bakker, P.A.H.M., Pieterse, M.J. (1998). Systemic resistance induced by rhizosphere bacteria. Ann. Rev. Phytopathol., 36, 453-483.

Verma, H. N., Srivastava, S., Varsha, Kumar, D. (1996), Induction of systemic resistance in plants against viruses by a basic protein from *Clerodendrum aculeatum* leaves, Phytopathol., 86, 485-492.

Walters, D., Walsh, D., Newton, A., Lyon, G. (2005). Induced resistance for plant disease control: Maximizing the efficacy of resistance elicitors. Phytopathology, 95, 1368-1373.

Yemm, E.W., Cocking, E.C. (1955). The determination of amino acids with ninhydrin. Analyst, 8, 209-230.

Yemm, E.W., Willis, A.J. (1954). The estimation of carbohydrates in plant extracts by anthrone. Biochemical J., 57, 508-514.