



International Journal of  
**Dairy Science**

ISSN 1811-9743



Academic  
Journals Inc.

[www.academicjournals.com](http://www.academicjournals.com)

## Antiviral Activity of Lactoferrin against *Potato virus x* *In vitro* and *In vivo*

<sup>1</sup>Soad H. Taha, <sup>2</sup>Samah A. Mokbel, <sup>1</sup>Mahmoud Abdel-Hamid and <sup>2</sup>Ali H. Hamed

<sup>1</sup>Department of Dairy Science, Faculty of Agriculture, Cairo University, Egypt

<sup>2</sup>Department of Virus and Phytoplasma Research, Plant Pathology Research Institute, Agricultural Research Center, Egypt

*Corresponding Author: Soad H. Taha, Department of Dairy Science, Faculty of Agriculture, Cairo University, Egypt*

### ABSTRACT

Potato is one of the most important food crops after wheat and rice. The effectiveness of lactoferrin against *Potato virus x* (PVX) *in vitro* and *in vivo* have been evaluated. Four concentrations of lactoferrin 100, 250, 500 and 1000 mg L<sup>-1</sup> were examined either *in vitro* culture medium or *in vivo* (greenhouse). The presence of the virus was evaluated by ELISA technique. Data demonstrated that application of 1000 mg L<sup>-1</sup> lactoferrin by spraying or combined with tissue culture proved to be an effective method for PVX-inhibition as compared with other concentrations. Also, results of antiviral activity of lactoferrin at concentration 1000 mg L<sup>-1</sup> showed great potential as phytotherapeutic source to produce quality and health plantlets for rapid and large scale *in vitro* production. Lactoferrin seemed to be very successful and inexpensive in controlling viral infection through *in vitro* technique or by spraying the infected and/or healthy plant under greenhouse condition.

**Key words:** Lactoferrin, *Potato virus x*, tissue culture, greenhouse, antiviral activity

### INTRODUCTION

Potato (*Solanum tuberosum* ssp. *tuberosum* L.) is the world's third most important food crop after wheat and rice which is roughly half the world's annual output of all root and tuber crops that estimated by 330 MT (Patil *et al.*, 2012). In Egypt, the annual potato production reached 4.8 Mt in 2013 making Egypt Africa's number one in the potato production (FAO., 2014). However, the potential production could exceed more than a quarter through control the diseases that reduce the yield (Agrios, 2005).

*Potato virus x* is a plant pathogenic virus of the family Alphaflexiviridae and the order Tymovirales. It is the type species of the genus *Potexvirus*. PVX is found mainly in potatoes causing mild or no symptoms in most potato varieties. There are no insect or fungal vectors known for this virus. PVX is the widespread wherever potato is grown and often completely infects certain commercial stocks, causing yield reductions (Burrows and Zitter, 2005).

During the last few years, important advances in virus chemotherapy were studied. Varieties of these antiviral agents affect viral replication or inhibit the virus specific events that occur during viral maturation and assembly (Streissle *et al.*, 1985).

Lactoferrin (Lf) is an iron-binding glycoprotein of the transferrin family, with a molecular mass of about 80 kDa. It presents in almost all mammalian secretions and in neutrophils which plays an important role as a modulator component of the immune system (Valenti *et al.*, 2004;

Legrand *et al.*, 2005; Gonzalez-Chavez *et al.*, 2009). Its concentration in the milk varies from 7 g L<sup>-1</sup> in the colostrum (first milk) to 1 g L<sup>-1</sup> in mature milk. Human colostrum has the highest concentration, followed by human milk, then cow milk (150 mg L<sup>-1</sup>) (Sanchez *et al.*, 1992).

A variety of biological properties have been ascribed for Lf, including antibacterial, antiviral, antifungal, antiparasitic, anticarcinogenic activities, anti-inflammatory and antitumor effects (Legrand *et al.*, 2008; Baker and Baker, 2009; Taha *et al.*, 2010; Florian *et al.*, 2012; Conneely, 2001). Some of these depend on the iron-chelating capacity of Lf; others are related to its ability to interact with molecular and cellular components of both host and pathogens.

Lactoferrin used as recombinant protein in different transgenic plant systems for variety of applications including tobacco (Choi *et al.*, 2003), potato (Chong and Langridge, 2000), tomato (Lee *et al.*, 2002), maize (Samyn-Petit *et al.*, 2001), barley (Kamenarova *et al.*, 2007), ginseng (Kwon *et al.*, 2003) or commercially produced from transgenic rice (Suzuki *et al.*, 2003). Also, it used as antiviral against some plant diseases i.e., *Tomato yellow leaf curl virus* (Abdelbacki *et al.*, 2010) or *Tobacco mosaic virus* in tobacco seedlings (Wang *et al.*, 2012, 2013).

The objective of this study was to examine the antiviral activity of native lactoferrin against *Potato virus x*, the most important virus that severely affects potato crop and productivity in Egypt, using tissue culture technique and spraying the plants in greenhouse by the aqueous solution of lactoferrin.

## MATERIALS AND METHODS

- Lactoferrin (LF) was kindly obtained from Armor Proteins (France)
- ELISA kits were purchased from LOEWE Biochemica, GmbH, DSMZ (Germany)
- Culture medium was obtained from CAISSON (USA) with macronutrients, micronutrients, vitamins and glycine as described by Murashige and Skoog (1962)
- Agar-agar powder [as a solidifying agent for culture medium] was supplied by Sigma-Aldrich (Germany)
- All other chemicals used were of analytical grade
- Healthy potato seeds were obtained from Ministry of Agriculture and Land Reclamation, Central Administration for seed Production, Giza, Egypt

**Source of virus:** Samples from naturally infected potato plants exhibiting viral infection were collected from Al-Behera Governorate and directly transferred to the laboratory for detection. The observed symptoms included mild mosaic and crinkle on leaves.

### Isolation and identification

**Selective host plants and symptomatology:** About 2 g of naturally infected potato leaf tissues were grounded in 0.01 M phosphate buffer, containing 0.2% Diethyldithiocarbamate, at pH 7.2 then, mechanically transmitted to each three seedlings of the following host plant, *Gomphrena globosa* L., *Chenopodium amaranticolor* L. and *Chenopodium quinoa* wild grown in clay pots containing sterilized soil and kept in an insect-proof greenhouse. Four weeks later, seedlings were examined for symptoms expression by visual inspection and DAS-ELISA.

The virus isolate was biologically purified from the single local lesion as reported by Kuhn (1964) produced on *G. globosa*. After successive signal local lesion transfers in the local lesion host, the resulting virus isolate was propagated in *N. tabacum* cv. White Burley plants.

The sap from systemically PVX-infected *N. tabacum* cv. White Burley were inoculated onto healthy potato leaves slightly dusted with carborundum 600 mesh and served as a source for *in vitro* and *in vivo* experiments.

**Serological detection:** Enzyme linked immune-sorbent assay (DAS-ELISA) was conducted to test the isolated virus against PVX according to Clark and Adams (1977). Observation was measured by ELISA-Reader at 405 nm. Positive result signed if the tested samples had average point more than 2x point of negative sample. All tests were carried out in the serological laboratory of Virus and Phytoplasma Research Department, Plant Pathology Research Institute, ARC. Plants reacted positively against PVX antiserum were served as source of virus inoculums.

**Effect of lactoferrin on PVX *in vitro*:** Explants of 2-3 cm in length were collected from one month old potato plants after inoculation that being maintained in the greenhouse and then were surface sterilized with 20% commercial Clorox solution and one drop of tween 20 and left for 20 min then rinsed 3 times with sterile distilled water for 5 min each.

The surface sterilized explants were cut into single nodal segments and cultured in sterilized culture jar containing 25 mL modified solid MS medium according to Murashige and Skoog (1962), supplemented with 30 g L<sup>-1</sup> sucrose and 9 g L<sup>-1</sup> agar without any additions of growth regulators and then kept in a growth chamber. The established *in vitro* plantlets were then sub-cultured every three weeks using single node cuttings to build up the stock plants necessary for the experiment. Four levels of lactoferrin powder 100, 250, 500 and 1000 mg were applied and solid medium was replaced with liquid one. Each level was dissolved in 100 mL of autoclaved liquid culture medium and filter sterilized before adding to 900 mL of the culture medium inside a laminar flow, then left for 48 h. Ten infected plantlets with PVX were sub-cultured on filter paper bridge in liquid medium supplemented with each level of lactoferrin. The percentage of virus-free plantlets was indexed using DAS-ELISA test after 21 days.

A complete randomize design was used for analysis all data with three replications. The treatment means were compared by least significant difference (L.S.D) test as given by Snedecor and Cochran (1994).

All experiments were conducted under aseptic condition in laminar flow and cultures at all growth stages were incubated under artificial conditions 22/18°C day/night temperatures and 16 h photoperiod for three weeks.

#### **Effect of lactoferrin on PVX *in vivo***

**Pre-inoculation treatment:** Each concentration of lactoferrin was sprayed on the tested plants which were mechanically inoculated separately with PVX infected sap at different intervals: 1, 3, 5 and 7 days, respectively. Distilled water was used as a control. Twenty plants were used for each treatment. The inhibition percentages of virus were assessed firstly on the basis of symptom expression and then examined using DAS-ELISA.

**Post-inoculation treatment:** In this experiment, virus infected sap was applied first followed by lactoferrin treatment after 7 days. Distilled water was used as a control. Each treatment was conducted twice and twenty plants were used for each treatment. Samples of leaves were collected from each treatment separately after 4 weeks from inoculation, then examined using DAS- ELISA to determine the antiviral activity of lactoferrin against PVX.

## RESULTS AND DISCUSSION

**Isolation and identification:** As shown in Fig. 1a samples collected from naturally infected potato plants showed mild mosaic or crinkle on potato leaves which is in accordance with symptoms previously described for PVX infection on potato plants (Muhammad *et al.*, 2012).

Sap of naturally infected potato leaf tissues were mechanically inoculated into tested plants. After biological purification, the virus isolate was re-inoculated onto the selective host plants. Three plant species belonging to two families (Amaranthaceae and Chenopodiaceae) were mechanically inoculated with PVX, showed only local lesions on *Gomphrena globosa* L. after one month and serologically reacted against PVX antiserum while, no symptoms appeared on Chenopodiaceae family (*Chenopodium amaranticolor* and *Chenopodium quinoa*) and no serological reaction was detected against PVX antiserum. The local lesion host of PVX was propagated in *Nicotiana tabacum* cv. White Burley plants and showed mosaic on leaves after one month of incubation period under greenhouse conditions. Similar results were also recorded by El-Araby *et al.* (2009). The previously obtained PVX was mechanically transmitted to healthy potato plants (Fig. 1b) showed mosaic and crinkle on leaves (Fig. 1c and d).

**Therapeutic effect of lactoferrin against PVX:** Experiments were conducted *in vitro* and *in vivo*. The goal of *in vitro* phase of our study is to simulate the vegetative propagation process which is used to commercially production of potato plantlets throughout the year and to identify the most effective lactoferrin concentration on the virus-free plantlets production or the growth of plant as well as to evaluate the potential antiviral effects of different concentrations of lactoferrin by spraying the inoculated potato plants.

It was clear that the treatment with concentration 1000 mg L<sup>-1</sup> of lactoferrin is more distinguished for antiviral activity as compared to the other concentrations which increased the percentages of PVX-free potato plantlets to 80% *in vitro* (Table 1) or 70 and 50% under greenhouse condition (Table 2) with significant differences between treatments (lactoferrin concentrations). Also, the *in vitro* treatment was of high efficiency when compared with lactoferrin based-spraying which may be due to the loss of some amount of dissolved lactoferrin in the air.

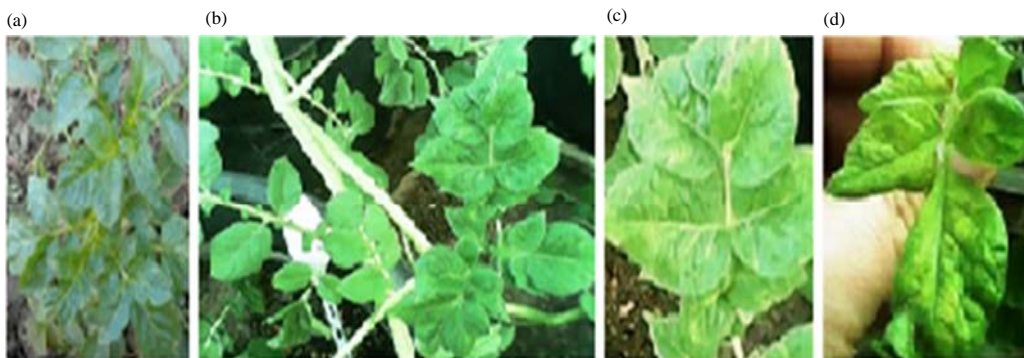


Fig. 1(a-d): (a) Naturally infected potato plants, (b) Healthy potato plants, (c) PVX-developed mosaic symptom and (d) Crinkle on leaves after mechanical inoculation of potato plants under greenhouse condition

Table 1: Effect of lactoferrin on production of PVX-free plantlets *in vitro*

Lactoferrin conc. (mg L <sup>-1</sup> )	Infected (%)	Healthy (%)
Control (Water)	100 <sup>a</sup>	0 <sup>d</sup>
100	90 <sup>ab</sup>	10 <sup>cd</sup>
250	85 <sup>b</sup>	15 <sup>c</sup>
500	50 <sup>c</sup>	50 <sup>b</sup>
1000	20 <sup>d</sup>	80 <sup>a</sup>

Ten plants/treatment-Data is based on DAS-ELISA detection

Table 2: Effect of lactoferrin treatments on post-inoculated potato plants by PVX under greenhouse condition

Lactoferrin conc. (mg L <sup>-1</sup> )	Infected (%)	Healthy (%)
Control (Water)	100 <sup>a</sup>	0 <sup>c</sup>
100	95 <sup>a</sup>	5 <sup>c</sup>
250	95 <sup>a</sup>	5 <sup>c</sup>
500	70 <sup>b</sup>	30 <sup>b</sup>
1000	30 <sup>c</sup>	70 <sup>a</sup>

Twenty plants/treatment-Data is based on DAS-ELISA detection

Table 3: Effect of pre-treatments of lactoferrin on PVX infectivity *in vivo*

Lactoferrin conc. (mg L <sup>-1</sup> )	Healthy (%)				Infected (%)				Inhibition (%)			
	1	3	5	7	1	3	5	7	1	3	5	7
Control (Water)	0 <sup>n</sup>	0 <sup>n</sup>	0 <sup>n</sup>	0 <sup>n</sup>	20 <sup>a</sup>	20 <sup>a</sup>	20 <sup>a</sup>	20 <sup>a</sup>	0 <sup>m</sup>	0 <sup>m</sup>	0 <sup>m</sup>	0 <sup>m</sup>
100	0 <sup>n</sup>	0 <sup>n</sup>	2 <sup>l</sup>	1 <sup>m</sup>	20 <sup>a</sup>	20 <sup>a</sup>	18 <sup>c</sup>	19 <sup>b</sup>	0 <sup>m</sup>	0 <sup>m</sup>	10 <sup>k</sup>	5 <sup>l</sup>
250	2 <sup>l</sup>	4 <sup>j</sup>	7 <sup>i</sup>	3 <sup>k</sup>	18 <sup>c</sup>	16	13 <sup>f</sup>	17 <sup>d</sup>	10 <sup>k</sup>	20 <sup>i</sup>	35 <sup>h</sup>	15 <sup>j</sup>
500	10 <sup>h</sup>	14	14	13 <sup>f</sup>	10 <sup>g</sup>	6 <sup>j</sup>	6 <sup>j</sup>	7 <sup>i</sup>	50 <sup>j</sup>	70 <sup>d</sup>	70 <sup>d</sup>	65
1000	12 <sup>g</sup>	17 <sup>c</sup>	18 <sup>b</sup>	15 <sup>d</sup>	8 <sup>h</sup>	3 <sup>i</sup>	2 <sup>m</sup>	5 <sup>k</sup>	60 <sup>f</sup>	85 <sup>b</sup>	90 <sup>a</sup>	75 <sup>c</sup>

Twenty plants/treatment-Data is based on DAS-ELISA detection

The antiviral activity of lactoferrin or its lobes against intracellular viral particles is still unclear. Generally, lactoferrin is a polypeptide chain and a number of studies have implicated these peptides that mainly derived from the N-lobe, to be responsible for antiviral role through its ability to interact with the viral molecules (Siciliano *et al.*, 1999) or based on the protein cationicity and  $\alpha$ -helical structure of lactoferrin (Lin *et al.*, 2011; Zhang *et al.*, 2013). Furthermore, lactoferrin has been described as an antiviral agent that affects a broad range of RNA and DNA viruses that infect humans and animals (Gonzalez-Chavez *et al.*, 2009) and plays a central role in the immune system of the body; in spite of plant systems are far less likely to harbor microbes pathogenic to humans than mammalian cells but one of the major advantages of plants is that they possess an endomembrane system and secretory pathway that are similar to mammalian cells (Vitale and Pedrazzini, 2005).

These hypotheses confirm the results of DAS-ELISA assays to monitor the effect of anti-PVX therapy supported the therapeutic evaluation of lactoferrin on the *in vitro* PVX that may explain the antiviral activity in the current study through application of lactoferrin in culture media as evident by no variation or change in the percentages of healthy potato plants after the first therapeutical trials or reconfirmed one. Finally, it is worthy to note that lactoferrin must be added after autoclaving the culture medium through sterilized filter to avoid losing of its antiviral activity by heat (Van der Strate *et al.*, 2001).

**Preventive effect of lactoferrin against PVX:** The goal of this part of study is to decrease the injury of infected potato plants through spraying different concentrations of lactoferrin under greenhouse condition. Data presented in Table 3 showed also that the most effective pre-inoculation treatment was 1000 mg L<sup>-1</sup> of lactoferrin as compared to the other concentrations. It is clear that the preventive treatment based on spray to be necessary every 3 or 5 days which



lead to increase the inhibition percentages to 85 or 90%, respectively with significant differences between lactoferrin concentrations. A negative reaction after 7 days where all inhibition percentages reduced again in each treatment even concentration 1000 mg L<sup>-1</sup> of lactoferrin.

According to the literatures, techniques using milk are frequently used in nurseries to stop the spread of virus between susceptible hosts when people touch the plant, during pruning and the inhibitory effects of milk were restricted by reducing the plant's susceptibility to the virus. These effects were on the virus and not on the plant (Gillian, 2005). Different modes of action of milk-based sprays were provided by Bettiol (1999), included an increase in the pH of the leaf surface (Ziv and Zitter, 1992), the establishment of a protective barrier (Mucharromah and Kuc, 1991; McGrath and Shishkoff, 1999) and the direct induction of systemic resistance (Reuveni *et al.*, 1993). Many studies have shown that at least part of the antiviral properties of milk can be attributed to a direct antiviral activity of lactoferrin (Abdelbacki *et al.*, 2010).

The interpretation of the application of lactoferrin to control viral infection is based on preventing virus entry into cells by the fact that it binds to the envelope virus protein (Yi *et al.*, 1997) or binds to cell-surface molecules that virus use either as receptors or co-receptors (Van der Strate *et al.*, 2001). The interaction of lactoferrin with viral envelope proteins or with receptors on cell surface is critical to blocking viral entry to target cells and infection is stopped at an early stage (Ward *et al.*, 2005). Therefore, lactoferrin not only prevents infection but also induces some type of resistance that maintains antiviral effects after the virus has entered the cell and inhibits the proliferation or replication of viruses (Abdelbacki *et al.*, 2010).

**Effect of lactoferrin on plant growth:** Increasing the concentration of lactoferrin had a positive influence on the performance proliferation of potato plantlets or could induce different multiple shooting responses without affecting the survival percentage where best results were obtained with MS media containing 1000 mg L<sup>-1</sup> of lactoferrin due to the possession of activities of cytokines or modulates a variety of cellular functions and increase cytokine response (Wakabayashi *et al.*, 2004). So, it is capable to penetrate a cell and speed up the process of cell division and thus has a profound effect on shoot formation or the proliferation of cells and act as growth factor activator "growth regulator" (Yanaihara *et al.*, 2000).

## CONCLUSION

Lactoferrin seemed to be very successful and inexpensive in controlling viral infection through *in vitro* technique or by spraying the infected and/or healthy plant under greenhouse condition and addition of lactoferrin in the culture media to be effective for enhancing the development of cultured explants. So it can be concluded that more attention must be focus on lactoferrin with high level (1000 mg L<sup>-1</sup>) in several crops through tissue culture technique to optimize regeneration of plantlets. Moreover, lactoferrin is a natural compound that allowing safe use based-spray in greenhouse cultivation thus avoiding the use of chemical compounds to immunize and protect plants against viral infection.

## ACKNOWLEDGMENT

The authors thank Armor Proteins (France) for their kindly provided offers.

## REFERENCES

- Abdelbacki, A.M., S.H. Taha, M.Z. Sitohy, A.I.A. Dawood, M.M.A.E. Hamid and A.A. Rezk, 2010. Inhibition of Tomato Yellow Leaf Curl Virus (TYLCV) using whey proteins. *Viol. J.*, Vol. 7. 10.1186/1743-422X-7-26.

- Agrios, G.N., 2005. Plant Pathology. 4th Edn., Academic Press, London, UK., pp: 25-37.
- Baker, E.N. and H.M. Baker, 2009. A structural framework for understanding the multifunctional character of lactoferrin. *Biochimie*, 91: 3-10.
- Bettiol, W., 1999. Effectiveness of cow's milk against zucchini squash powdery mildew (*Sphaerotheca fuliginea*) in greenhouse conditions. *Crop Protect.*, 18: 489-492.
- Burrows, M.E. and T.A. Zitter, 2005. Virus problems in potatoes. Vegetable MD Online, Department of Plant Pathology, Ithaca, New York. [http://vegetablemdonline.ppath.cornell.edu/ewsArticles/Potato\\_Virus.htm](http://vegetablemdonline.ppath.cornell.edu/ewsArticles/Potato_Virus.htm).
- Choi, S.M., O.S. Lee, S.Y. Kwon, S.S. Kwak, D.Y. Yu and H.S. Lee, 2003. High expression of a human lactoferrin in transgenic tobacco cell cultures. *Biotechnol. Lett.*, 25: 213-218.
- Chong, D.K.X. and W.H.R. Langridge, 2000. Expression of full-length bioactive antimicrobial human lactoferrin in potato plants. *Transgenic Res.*, 9: 71-78.
- Clark, M.F. and A.N. Adams, 1977. Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. *J. Gen. Virol.*, 34: 475-483.
- Conneely, O.M., 2001. Antiinflammatory activities of lactoferrin. *J. Am. Coll. Nutr.*, 20: 389S-395S.
- El-Araby, W.S., I.A. Ibrahim, A.A. Hemeida, A. Mahmoud, A.M. Soliman, A.K. El-Attar and H.M. Mazyad, 2009. Biological, serological and molecular diagnosis of three major potato viruses in Egypt. *Int. J. Virol.*, 5: 77-88.
- FAO., 2014. Potato production in Egypt. Food and Agriculture Organization (FAO), Rome, Italy.
- Florian, P.E., M. Moisei, M. Trif, R.W. Evans and A. Roseau, 2012. Antiinflammatory and anti-tumoral activity of human recombinant lactoferrin. *Rom. J. Biochem.*, 49: 163-171.
- Gillian, F., 2005. Milk as a management tool for virus diseases. Ministry of Agriculture, Food and Rural Affairs, November 2005. <http://www.omafra.gov.on.ca/english/crops/hort/news/grower/2005/11gn05a1.htm>.
- Gonzalez-Chavez, S.A., S. Arevalo-Gallegos and Q. Rascon-Cruz, 2009. Lactoferrin: Structure, function and applications. *Int. J. Antimicrob. Agents*, 4: 301.e1-301.e8.
- Kamenarova, K., K. Gecheff, M. Stoyanova, Y. Muhovski, H. Anzai and A. Atanassov, 2007. Production of recombinant human lactoferin in transgenic barley. *Biotechnol. Biotechnol. Equip.*, 21: 18-27.
- Kuhn, C.W., 1964. Separation of cowpea virus mixtures. *Pytopathology*, 54: 739-740.
- Kwon, S.Y., S.H. Jo, O.S. Lee, S.M. Choi, S.S. Kwak and H.S. Lee, 2003. Transgenic ginseng cell lines that produce high levels of a human lactoferrin. *Planta Med.*, 69: 1005-1008.
- Lee, T.J., P.P. Coyne, T.E. Clemente and A. Mitra, 2002. Partial resistance to bacterial wilt in transgenic tomato plants expressing antibacterial lactoferrin gene. *J. Am. Soc. Horticult. Sci.*, 127: 158-164.
- Legrand, D., A. Pierce, E. Ellass, M. Carpentier, C. Mariller and J. Mazurier, 2008. Lactoferrin structure and functions. *Adv. Exp. Med. Biol.*, 606: 163-194.
- Legrand, D., E. Ellass, M. Carpentier and J. Mazurier, 2005. Lactoferrin: A modulator of immune and inflammatory responses. *Cell Mol. Life Sci.*, 62: 2549-2559.
- Lin, Q., D. Fang, X. Hou, Y. Le and J. Fang *et al.*, 2011. HCV peptide (C5A), an amphipathic  $\alpha$ -helical peptide of hepatitis virus C, is an activator of N-formyl peptide receptor in human phagocytes. *J. Immunol.*, 186: 2087-2094.
- McGrath, M.T. and N. Shishkoff, 1999. Evaluation of biocompatible products for managing cucurbit powdery mildew. *Crop Protect.*, 18: 471-478.
- Mucharromah, E. and J. Kuc, 1991. Oxalate and phosphates induce systemic resistance against diseases caused by fungi, bacteria and virus in cucumber. *Crop Protect.*, 10: 265-270.



- Muhammad, N., S.H. Syed, B. Asia, H. Mumtaz and I. Babar *et al.*, 2012. ELISA-based detection of major potato viruses in tissue culture produced potato germplasm. *Int. J. Agric. Sci.*, 2: 75-80.
- Murashige, T. and F. Skoog, 1962. A revised medium for rapid growth and bio assays with tobacco tissue cultures. *Physiol. Plant.*, 15: 473-497.
- Patil, V.U., J. Gopal and B.P. Singh, 2012. Improvement for bacterial wilt resistance in potato by conventional and biotechnological approaches. *Agric. Res.*, 1: 299-316.
- Reuveni, M., V. Agapov and R. Reuveni, 1993. Induction of systemic resistance to powdery mildew and growth increase in cucumber by phosphates. *Biol. Agric. Horticult.*, 9: 305-315.
- Samyn-Petit, B., V. Gruber, C. Flahaut, J.P. Wajda-Dubos and S. Farrer *et al.*, 2001. N-glycosylation potential of maize: The human lactoferrin used as a model. *Glycoconjugate J.*, 18: 519-527.
- Sanchez, L., M. Calvo and J.H. Brock, 1992. Biological role of lactoferrin. *Arch. Dis. Childhood*, 67: 657-661.
- Siciliano, R., B. Rega, M. Marchetti, L. Seganti, G. Antonini and P. Valenti, 1999. Bovine lactoferrin peptidic fragments involved in inhibition of herpes simplex virus type 1 infection. *Biochem. Biophys. Res. Commun.*, 264: 19-23.
- Snedecor, C.W. and W.G. Cochran, 1994. *Statistical Method*. Iowa State University Press, Ames, Iowa, USA.
- Streissle, G., A. Paessens and H. Oediger, 1985. New antiviral compounds. *Adv. Virus Res.*, 30: 83-138.
- Suzuki, Y.A., S.L. Kelleher, D. Yalda, L. Wu, J. Huang, N. Huang and B. Lonnerdal, 2003. Expression, characterization and biologic activity of recombinant human lactoferrin in rice. *J. Pediatr. Gastroenterol. Nutr.*, 36: 190-199.
- Taha, S.H., M.A. Mehrez, M.Z. Sitohy, A.G.A. Dawood, M.M.A.E. Hamid and W.H. Kilany, 2010. Effectiveness of esterified whey proteins fractions against Egyptian Lethal Avian Influenza a (H5N1). *Virol. J.*, Vol. 7. 10.1186/1743-422X-7-330
- Valenti, P., F. Berlutti, M.P. Conte, C. Longhi and L. Seganti, 2004. Lactoferrin functions: Current status and perspectives. *J. Clin. Gastroenterol.*, 38: S127-S129.
- Van der Strate, B.W.A., L. Beljaars, G. Molema, M.C. Harmsen and D.K.F. Meijer, 2001. Antiviral activities of lactoferrin. *Antiviral Res.*, 52: 225-239.
- Vitale, A. and E. Pedrazzini, 2005. Recombinant pharmaceuticals from plants: The plant endomembrane system as bioreactor. *Mol. Intervent.*, 5: 216-225.
- Wakabayashi, H., M. Kurokawa, K. Shin, S. Teraguchi, Y. Tamura and K. Shiraki, 2004. Oral lactoferrin prevents body weight loss and increases cytokine responses during herpes simplex virus type 1 infection of mice. *Biosci. Biotechnol. Biochem.*, 68: 537-544.
- Wang, J., H.Y. Wang, X.M. Xia, P.P. Li and K.Y. Wang, 2013. Inhibitory effect of esterified lactoferin and lactoferrin against Tobacco Mosaic Virus (TMV) in tobacco seedlings. *Pesticide Biochem. Physiol.*, 105: 62-68.
- Wang, J., Y.K. Zhu, H.Y. Wang, H. Zhang and K.Y. Wang, 2012. Inhibitory effects of esterified whey protein fractions by inducing chemical defense against Tobacco mosaic virus (TMV) in tobacco seedlings. *Ind. Crops Products*, 37: 207-212.
- Ward, P.P., E. Paz and O.M. Conneely, 2005. Multifunctional roles of lactoferrin: A critical overview. *Cell. Mol. Life Sci.*, 62: 2540-2548.

- Yanaihara, A., Y. Toma, H. Saito and T. Yanaihara, 2000. Cell proliferation effect of Lactoferrin in human endometrial stroma cells. *Mol. Hum. Reprod.*, 6: 469-473.
- Yi, M., S. Kaneko, D.Y. Yu and S. Murakami, 1997. Hepatitis C virus envelope proteins bind lactoferrin. *J. Virol.*, 71: 5997-6002.
- Zhang, J., A. Mulvenon, E. Makarov, J. Wagoner and J. Knibbe *et al.*, 2013. Antiviral peptide nanocomplexes as a potential therapeutic modality for HIV/HCV co-infection. *Biomaterials*, 34: 3846-3857.
- Ziv, O. and T.A. Zitter, 1992. Effects of bicarbonates and film-forming polymers on cucurbit foliar diseases. *Plant Dis.*, 26: 513-517.