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# Comparative studies on eco-friendly management of tomato leaf curl using cow, goat and buffalo's milk whey protein

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ABSTRACT

The experiments were conducted to know the effect of milk whey protein on tomato leaf curl disease. For comparative studies, milk of cow, goat and buffalo was used. Trial fields which were treated with milk whey proteins showed less disease incidence as compared to the trial field which was not treated. Studies also showed that buffalo's milk had highest inhibitory effect against tomato leaf curl followed by cow and goat's milk. These investigations shows that milk whey protein could be used as an eco-friendly measure to control tomato leaf curl disease.

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## INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill.) of family Solanaceae is one of the important vegetable crops grown throughout the world. UP is one of the major tomato growing states of India. The most widespread and destructive disease of tomato is tomato leaf curl, as sometimes it leads to cent percent crop loss (Butter and Rataul, 1981, Ansari *et al.*, 2005). The tomato leaf curl virus (TLCV) is transmitted by whitefly (*Bemisia tabaci*, Genn.) (Vasudeva and Somaraj, 1948).

In order to control TLC, farmers normally use chemical insecticides which leads to serious consequences affecting human physiology on consumption of such fruits. The use of insecticides also disturbs the ecobalance of soil and the environment by killing non targeted insects, which harbour beneficial parasites and predators. So an alternative eco-friendly method is necessary to avoid the undesirable effects of the insecticides. In 1940s several investigators suggested the use of milk as spraying or dipping of seedlings for reducing the incidence of virus infections. Recent studies demonstrated the effectiveness of milk in reducing infection of tobacco mosaic virus (TMV) in pepper, tomato, and tobacco.

There are two major categories of milk protein that are broadly defined by their chemical composition and physical properties. The casein family contains phosphorus and will coagulate or precipitate at pH 4.6. The serum (whey) proteins do not contains phosphorus, and these proteins remain in solution in milk at pH 4.6. Out of total protein content of milk, 80 per cent is casein protein and 20 per cent is whey protein. The main constituents of whey are a-lactalbumin (ALA), b-lactoglobulin (BLG) and two small globular proteins that account for approximately 70-80 per cent of total whey protein. The biological components of whey proteins, including b-lactoglobulin, a-lactalbumin, lactoferrin, lactoperoxidase, immunoglobulins and glycomacropeptides, demonstrate a wide range of immune enhancing properties, and act as antioxidant, antihypertensive, antitumer, antiviral, antimicrobial and chelating agent. The objective of the present work is to study the comparative inhibition of tomato leaf curl disease using cow,goat and buffalo's milk.

## MATERIALS AND METHODS

Simplified short timed method for the esterification of milk protein is used for esterification of whey protein fraction using >99.5 per cent methyl alcohol, at 4°C for 10 h. as follows:

Native whey proteins fractions were dispersed (5%, w/v) in methyl alcohol 99.5 per cent. Amounts of hydrochloric acid equivalent to 50 molar ratio of acidity (MR, mole acid/mole carboxyl group) were added drop-wise at the start of the reaction time. All the reaction mixtures were kept at 4°C under continuous stirring. At the end of the reaction (6 h), the samples

were centrifuged at 10000 g for 10 min. The resulting supernatant was discarded and the residue was dispersed in a volume of alcohol (99.7% ethanol) equal to that of the discarded supernatant, and well mixed before re-centrifuging at the same conditions. This washing step was repeated three times. The final precipitate was dissolved in an appropriate amount of distilled water then submitted to freeze-drying. The lyophilized samples were kept at -20°C until analysis. The colour reaction using hydroxylamine hydrochloride was used to quantify the extent of esterification of proteins.

Tomato plants were planted under green house conditions taking into consideration all the environmental requirements conditions of irrigation, fertilization etc. 20days old seedlings were transplanted in four different trial fields. Plants of field 1 were treated with cow milk, of field 2 with goat milk, of field 3 with buffalo milk and field 4 remain untreated, used as control. After 10, 20 and 30 days, the diseased and healthy plant counts were taken from $2x2m^2$ areas of five random places in each of four trial fields and per cent disease incidence (PDI) was calculated by the following formula:

$$PDI = \frac{P_1 \text{ X } 100}{P_2}$$

where, $P_1$ = Number of infected plants.
$P_2$ = Total number of plants.

## RESULTS AND DISCUSSION

The average disease incidence of field -3 was lowest among all followed by field-1, field-2 and field-4, indicating that buffalo's milk was the most effective inhibitor against tomato leaf curl followed by cow and goat's milk (Table 2). It is clear from the Table 1 that protein content in buffalo's milk is high so the whey protein content will also be higher than cow and goat's milk, consequently inhibitory effect of buffalo's milk was highest among all.

Early studies led to several hypotheses about mode of action of milk. The first one was in the 1930s suggested that milk inhibited infection by somehow reducing the plant's susceptibility to the virus. The second one in the 1940s suggested that the milk "inactivated" the virus by forming a loose "molecular union" which, if broken, results in re-activation of the virus. That is, the inhibiting effects were reversible and the effect was on the virus and not the plant. It was also found that the inhibitory effects were restricted only in the treated part of the plant. Furthermore, investigations suggested that the active substance in the milk was a protein. Milk whey proteins acquire net positive charges after esterification with methanol or ethanol enabling them to interact with negatively charged macro-molecules such as nucleic acids or some proteins . Consequently, these basic proteins may interact with viral DNA or RNA. Esterification not only increases the gross positive charge of the protein but also its hydrophobicity by grafting hydrophobic methyl or ethyl groups on the carboxyl groups of aspartyl and glutamyl residues. Enhanced hydrophobicity may also promote hydrophobic interactions with the hydrophobic binding sites formed by viral capsid proteins.

**Table 1: Comparisons of cow, goat and buffalo milk contents**

| Constituents | Unit | Cow | Goat | Buffalo |
|---|---|---|---|---|
| Water | g | 87.8 | 88.9 | 81.1 |
| Protein | g | 3.2 | 3.1 | 4.5 |
| Fat | g | 3.9 | 3.5 | 8.0 |
| Carbohydrate | g | 4.8 | 4.4 | 4.9 |
| Energy | kcal | 66 | 60 | 110 |
| Energy | kJ | 275 | 253 | 463 |
| Sugars (lactose) | g | 4.8 | 4.4 | 4.9 |
| Cholesterol | mg | 14 | 10 | 8 |
| Calcium | mg | 120 | 100 | 195 |
| Saturated fatty acids | g | 2.4 | 2.3 | 4.2 |
| Monounsaturated fatty acids | g | 1.1 | 0.8 | 1.7 |
| Polyunsaturated fatty acids | g | 0.1 | 0.1 | 0.2 |

**Table 2 : Inhibitory effect of cow, goat and buffalo milk whey protein on tomato leaf curl**

| Sr. No. | Treated with | | Per cent disease incidence | | |
|---|---|---|---|---|---|
| | | | After 10 days | After 20days | After 30 days |
| 1 | Cow milk | $T_1$ | 21.2 | 24.6 | 31.0 |
| | | $T_2$ | 23.0 | 25.8 | 28.5 |
| | | $T_3$ | 22.5 | 27.3 | 29.2 |
| | | $T_4$ | 22.3 | 26.0 | 29.4 |
| | | $T_5$ | 21.8 | 26.3 | 30.5 |
| 2 | Goat milk | $T_1$ | 27.6 | 30.4 | 34.9 |
| | | $T_2$ | 25.4 | 32.0 | 36.5 |
| | | $T_3$ | 28.1 | 31.5 | 34.8 |
| | | $T_4$ | 26.4 | 31.1 | 35.0 |
| | | $T_5$ | 27.9 | 30.8 | 35.7 |
| 3 | Buffalo milk | $T_1$ | 17.3 | 20.7 | 25.6 |
| | | $T_2$ | 20.1 | 22.1 | 26.4 |
| | | $T_3$ | 19.6 | 21.9 | 24.8 |
| | | $T_4$ | 18.9 | 21.3 | 24.2 |
| | | $T_5$ | 18.2 | 20.8 | 23.9 |
| 4 | Untreated | $T_1$ | 41.5 | 45.5 | 49.7 |
| | | $T_2$ | 39.9 | 48.2 | 54.3 |
| | | $T_3$ | 44.7 | 50.1 | 57.5 |
| | | $T_4$ | 40.7 | 46.4 | 56.5 |
| | | $T_5$ | 42.3 | 49.1 | 57.2 |

Finally the use of alternative "green" methods would have its advantage in the market, as many consumers are ready to pay more for pesticide-free products. This point could be of enough interest to justify the present work.

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