



## Homeopathic Medicines Protect Environment, Health and Development by Controlling Mulberry Diseases

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### Abstract

Plant diseases, caused by pathogens, significantly reduce food production particularly in the developing world where farmers have little knowledge of these pests. In sericulture, mulberry is an economical plant because silk production depends on the nutritive quality of the leaves which is hampered by various pathogen attacks like nematodes, fungus, virus, bacteria and insects etc. Recently, synthetic- and chemical- pesticides are the most effective means of control, but they are both expensive and environmentally unfriendly. The “evils” of synthetic- and chemical- pesticides has been a major concern to environmentalists. The use of chemical pesticides may achieve a measure of control of those mulberry diseases but there remains the problem of residual toxicity in the treated plants and this toxicity results in reduced palatability of the leaves to the feeding silkworm larvae, reduction in growth of the larvae and also in silk production. These are serious issues which directly cause crises of financial losses, food productions, and climatic changes, but in combination, their impact could be catastrophic for the global economy. To move forward will require new and more efficient solutions, technologies and products. Climate change and resource productive economies are now universally recognized as a significant global environmental challenge. To meet the challenge of the problems, a number of plant bio-nematicides though effective and easily biodegradable are not easily available in large quantities from natural sources and isolation of only a small quantity of an effective metabolites requires huge quantities of plant materials. This would result in rapid depletion of natural resources, particularly in tropical regions. Indiscriminate use of plant resources have already created problem of biodiversity conservation in the world. Bio-nematicides from animal origin (like nematode extract) reduce nematodes infestation in different plants and root callous by using their defense-response against nematode infection. But it remains as a problem.

To conquer this situation, the only ‘Homeopathy’ can solve all the above mentioned problems. Here, Homeopathic medicines; Cina, prepared from the flowering meristems of *Artemisia nilagirica* (Clarke) pamp and Aakashmoni, prepared from the funicles of *Acacia auriculiformis* A. Cunn, mixed with distilled water @ 7.2 mg/ml, were applied by foliar spray once daily for 15 days @ 10ml/plant on mulberry (*Morus alba* L., cv. S1) are highly effective in ameliorating mulberry diseases; root-knot [*Meloidogyne incognita* (Kofoid and White) Chitwood], leaf spot [*Cercosporam moricola* (Cooke)], powdery mildew [*Phyllactinia corylea* (Pers.) Karst], mosaic disease (mosaic virus) and tukra disease [*Maconellicoccus hirsutus* (Green)]. Both the drugs also improve the plant growth effectively which directly increase photosynthesis rate and significantly reduce CO<sub>2</sub> in the environment. Both the drugs also improve the growth of silkworms, shell weight, sex ratio percentage [SR%] and egg laying capacity of mother moth and also increase silk production and effective rate of silkworms rearing [ERR] commercially which directly enriches sericulture industry as well as agriculture sector. These cost-effective homeopathic medicines are easily available, biodegradable, non-phytotoxic and non-pollutant as well as conserve our biodiversity which will contribute towards “Sustainable Environment, Health and Development”.

**Keywords:** Homeopathic medicines; Cina; Aakashmoni; Control; Mulberry disease; Sericulture; Environment

### Introduction

Mulberry (*Morus alba* L.) is an important economical crop plant in sericulture and it grows under a wide range of ecological condition. It holds a special place as a major foreign exchange earner for many tropical and temperate countries. India secures the second position for the production of raw silk in the world, which is short about 30% to fulfill the home requirements [1]. The reasons for this deficiency as well as low quality of raw silk are, however, generally attributed to build up of the diseases of mulberry and silkworms, inadequate employment of improved culture and rearing practices [1,2-11]. Right from sprouting and throughout growing seasons, it is largely affected by a number of pathogens like plant parasitic nematodes, fungus, bacteria, virus and insects causing various diseases forming disease-complex and break the host resistance [1,2-12]. These pathogens are the main obstacles causing considerable loss in yield and nutritive value of mulberry foliage. Feeding of the diseased leaves affect the health of the silkworms adversely and the cocoon yield in terms of quality and quantity [2-11,13,14]. Root-knot disease, caused by *Meloidogyne incognita* (Kofoid and White) Chitwood, reduces 10-12% leaf yield in addition

to affecting the leaf quality for silkworms feeding [3-14]. Leaf spots disease caused by *Cercosporam moricola* (Cooke) fungus, losses 10-35% leaf yield reducing moisture, proteins adversely and ultimately the quality and quantity of cocoons. *Phyllactinia corylea* (Pers.) Karst fungus, causing powdery mildew disease, is the most common and wide spread economically important disease reducing 10-30% leaf yield and reducing the crude protein content by as much as 33%. The mosaic disease caused by mosaic virus results in inward curling of leaves, particularly leaf margins and tip with chlorotic lesions on the leaf surface, stunted growth and suppressed leaf size [1-14]. Tukra

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disease, caused by *Maconellicoccus hirsutus* (Green) (*Pseudococcidae*), tremendously reduces the leaves, depleting the nutritive value and plant growth, leaf yield and leaf protein content significantly [1-14].

Recently, synthetic and chemical pesticides are the most effective means of control, but they are both expensive and environmentally unfriendly. For sustainability of agriculture therefore, farmers should divorce the synthetic and chemical pesticides strategy and marry the phytochemicals option which is non-toxic to man and the environment, biodegradable and affordable to the peasant farmer in the developing world. The "evils" of synthetic and chemical pesticides has been a major concern to environmentalists. Recently efforts have therefore been shifted towards the use of plant extracts against pathogens as alternative to synthetic compounds. But it is not cost effective and it affects our biodiversity conservation directly [1-15].

To overcome this situation, it has been already observed that the extract prepared from the funicles of *Acacia auriculiformis* A. Cunn. and its pure compounds acaciasides (A&B), are effective in reducing mulberry diseases leaving no residual toxicity in the leaves to affect the growing silkworm larvae [2,3,16-18] And recently it has also been observed that the use of Cina, prepared from the flowering meristems of *Artemisia nilagirica* (Clarke) pamp and Aakashmoni prepared from the funicles of *Acacia auriculiformis* A. Cunn. on mulberry reduced root-knot disease and enriched sericulture industry [10].

## Aims and objectives

The purpose of the present investigation is to see the efficacy of the homeopathic medicines; Cina 200C, prepared from the flowering meristems of *Artemisia nilagirica* (Clarke) pamp and Aakashmoni 200C, prepared from the funicles of *A. auriculiformis*, in ameliorating root-knot disease of mulberry (*M. alba*, cv. S<sub>1</sub>) caused by *M. incognita* root-knot nematodes pathogens and also to find out if the Aakashmoni 200C can reduce the four foliar diseases, caused by pathogens, under field condition. The foliar diseases were: leaf spot disease caused by *Cercosporam moricola* (Cooke) fungus pathogens, powdery mildew disease caused by *Phyllactinia corylea* (Pers.) Karst fungus pathogens, mosaic disease caused by mosaic virus pathogens and tukra disease caused by *Maconellicoccus hirsutus* (Green) mealy bug pathogens. The effects of the leaves of the Cina 200C- and Aakashmoni 200C- treated plants on the leaf consumption, growth of silkworm's larvae, silk gland weight and effective rate of rearing (ERR) were also observed.

In course of our experiments with anti-nematode agents, Aakashmoni 200C, it was observed that the mulberry plants besides being infected with root-knot nematodes were also naturally infected with above mentioned four foliar diseases (leaf spot, powdery mildew, mosaic viral and tukra disease). Thus both the root-knot and foliar diseases, caused by various plant pathogens, were taken in to consideration during the evaluation of the effects of Aakashmoni 200C. The result would be more realistic in terms of the potentiality of the Aakashmoni 200C, use as potential bio-agents, in controlling various plant pathogens.

## Materials and Methods

### Site of the experimental plots

The field experiment was carried out at the Sriniketan Sericultural Composite Unit, Government of West Bengal, India where temperature was 28 ± 5°C and relative humidity was 75 ± 5%. Soil and root samples [19-22] were taken at random from a sericulture field spreading over

an area of 5.6 acre of land with a view to determining the extent and intensity of *Meloidogyne incognita* (Kofoid & White) Chitwood nematode pathogen infestation. Later, three areas (in the same locality and climatic condition) each measuring 0.02 has one naturally root-knot disease infected- untreated field and other two naturally root-knot disease infected Cina 200C and Aakashmoni 200C- treated field, were demarcated in the mulberry field where there were no soil differences as well as environmental factor.

The first area nematode infected (2863 ± 55 J<sub>2</sub>/1 kg of soil) sandy soil was mixed with yard manure (2: 1 vol/vol). Every day, at least 40 random sampling of moist rhizospheric soil (200g of soil i.e., each sample collected by making a hole of 1.8 cm wide and 6 cm deep) were done in the nematode infected area for 30 days and were assessed the *M. incognita* population [19,20] and this naturally infected soil-filled area, demarking untreated field, was replicated thrice.

The other two areas of naturally *M. incognita* infected sandy soil field was also prepared by mixing yard manure (2:1 vol/vol), removing weeds, irrigating water and interchanging among the soil for uniform distribution of manure and nematodes in the naturally infected field which was estimated by regular soil sampling like a same process of previous one. These naturally infected soil-filled areas, demarking treated fields, were also replicated thrice.

Mature three years old mulberry cutting, *Morus alba* L., cv. S<sub>1</sub> (average 25cm length and 20g fresh weight) collected from same sericulture field, were planted with a gap of 45cm throughout the experimental fields where there were no soil difference and climatic conditions. The planted mulberry cuttings were allowed to grow for a period of three months. Regular rhizospheric soil and root sampling (at random) were done for estimation of nematode population during this three month growth period of mulberry in all fields [19,20,23]. At least 80 random rhizospheric soil samples (200g in each sample) were collected from rhizospheric root-soil area of root (10-15cm X 10-15cm) and at least 40 random root samples (2g fresh root in each sample) were collected from newly formed roots (or gall roots) for determining the intensity or presence of nematodes in all the experimental fields [2,3,9].

After three months growth of mulberry, *M. incognita* populations were estimated in the rhizospheric soil as well as roots [19-22] (at least 40 at random sampling in each area) of mulberry plants in each areas of mulberry field. The *M. incognita* infected mulberry plants achieved growth of 50-60 cm in height. All the infected mulberry plants were divided into batches. The batches were; untreated- batches, Cina 200C- and Aakashmoni 200C- treated batches and each batch had 8-plots (20 plants/plot).

At first all the plants were pruned, manured with NPK and irrigated every 7 days. Rhizospheric soil was interchanged among the plants to keep the nematode infestation as uniform as possible in the naturally infected field. After pruning, the plants were allowed to grow for a period of 135 days when their root-knot, leaf spot, powdery mildew, viral and tukra diseases were assessed [1-3,24-26]. The field trial was replicated three times.

### Plant pathogens caused mulberry diseases

**Root-knot disease:** Rhizospheric soil and root sample were taken at random from all the infected plots. *Meloidogyne incognita* populations (10 samples/plot in each plant group) were estimated in

the rhizospheric soil as well as roots [2-9,19,20] of infected mulberry plants. Total number and surface area of leaves of all plant groups were counted [2,3,10]. Total number of root-galls/plant were counted in the infected roots of mulberry plants [2,23,27,28]. The total protein content of the leaf and root samples (10 at random sampling/plot) from plots was determined [27-29]. All the data from experiments were counted for statistical analysis by student's t- test. In this field trial, sacrifices of mulberry plants were not done due to well reported pathological characters from our previous experiments [2,3].

**Foliar diseases:** The main foliar diseases, observed in the sericulture field, were: leaf spot disease caused by *Cercosporam moricola* (Cooke) fungus pathogens, powdery mildew disease caused by *Phyllactinia corylea* (Pers.) Karst fungus pathogens, mosaic disease caused by mosaic virus pathogens and tukra disease caused by *Maconellicoccus hirsutus* (Green) mealy bug pathogens. All the disease identified according to their characteristic symptoms by the experts concerned [1-4,12,24]. Diseased leaves of each type were counted in each plot [30]. The percentage of disease infection was based on diseased leaf surface area [30-32].

#### Preparation of homeopathic mother tincture (MT)

Air-dried and powdered flowering meristems of *Artemisia nilagirica* (Clarke) Pamp- and funicles of *Acacia auriculiformis* A. Cunn, were extracted with 90% ethanol at room temperature ( $25 \pm 2^\circ\text{C}$ ) for 15 days and were filtered for collecting extract. Later, the ethanol from the extracts was removed by evaporation at room temperature ( $25 \pm 2^\circ\text{C}$ ). The residues were dried in a desecrator over anhydrous calcium chloride. The crude residues were dissolved in 90% ethanol at 1mg/ml concentration and were formed homeopathic mother tincture of *A. nilagirica* called Cina MT and *A. auriculiformis*, named Aakashmoni MT (Original solution or crude extract) respectively [7,10,11,14].

#### Preparation of potentized Liquid medicine

The homeopathic mother tinctures of Cina MT and Aakashmoni MT were diluted respectively with 90% ethanol (1:100) proportionate in a round vial. The vial were filled up to two-third of its space, tightly corked. And then were given 10 powerful down ward strokes of the arm. This process of mechanical agitation is called succession. This was the 1<sup>st</sup> centesimal potency named Cina 1C and Aakashmoni 1C. All the subsequent potencies were prepared by further diluting each potencies with 90% ethanol in the same proportion (1:100) and the mixture were given 10 powerful down ward strokes. In this way potencies up to Cina 200C and Aakashmoni 200C were prepared respectively [10].

#### Preparation of medicated globules

Both the homeopathic potencies in liquid form can be kept in globules. A vial were filled up to two-third of its empty space with sucrose globules of a particular size. Few drops of a liquid potency of Aakashmoni 200C were poured in to the vial to just moisten all the globules. The vial were corked and then shaken so that all globules were uniformly moistened. The cork was loosened and the vial was turned upside down to allow excess liquid drain out. After keeping the vial in the inverted position for nine to ten hours, the vial were turned upright, well corked and kept in a cool dry place away from light. The dry globules were then kept in a vial and medicated globules were known to retain their properties for many years. In this process the drug soaked globules Cina 200C and Aakashmoni 200C was prepared [10,11].

#### Preparation of control globules

A vial were filled up to two-third of its empty space with sucrose globules of a particular size. Few drops of 90% ethanol were poured in to the vial to just moisten all the globules. The vial were corked and then shaken so that all globules were uniformly moistened. The corks were loosened and the vial is turned upside down to allow excess liquid to drain out. After keeping the vial in the inverted position for nine to ten hours, the vial were turned upright, well corked and kept in a cool dry place away from light. The dry globules were then kept in a vial to retain their properties for many years. In this process the 90% ethanol soaked control sucrose globules were prepared. The control globules were prepared in the same way for comparison to the preparation of medicated Cina 200C- and Aakashmoni 200C- globules which were prepared with the 90% ethanol media [10,11].

#### Preparation of test- and control- solutions

The drug soaked globules of Cina 200C- and Aakashmoni 200C- were then be mixed with sterile distilled water in the proportion of 7.2 mg globules/ml of water [10,11]. The 90% ethanol soaked globules were then mixed with sterile distilled water in the proportion of 7.2 mg globules/ml of water and the Cina 200C- and Aakashmoni 200C- control solutions were prepared for comparison to the preparation of test solutions [10,11].

#### Mortality test

Three sets of cavity block with 1ml distilled water containing 50 larvae ( $J_2$ ) of *M. incognita* were taken; one set was treated as control and other two were treated as treatment sets of Cina 200C and Aakashmoni 200C. To assess the direct effect of Cina 200C- and Aakashmoni 200C- test solutions, the water was removed by pipette from all the treatment sets, and immediately replaced by 1ml of test solutions - Cina 200C and Aakashmoni 200C (7.2 mg globules/ml concentration) were added respectively. To assess the direct effect of control solution, the control set was received 1 ml of control solution and observed with every 30 minutes interval for a period of 12 hours exposure period at room temperature ( $25 \pm 2^\circ\text{C}$ ). This mortality test [33] was replicated five times. It was noted that both the control (without drugs) and treatment (with drugs) sets were received sucrose globules [10]. This mortality tests were replicated five times.

#### Treatment

Seventy six days after pruning, of mulberry plants, all the treatment were done by foliar spray @ 10ml/plant (7.2mg/ml concentration) once daily for 15 days with Cina 200C- and Aakashmoni 200C - test solutions and control solutions respectively. Treatments were given in such a way that all the leaves of the plants were completely sprayed with solutions. During spraying, the soil surface underneath each plant was covered with polyethylene sheet [2-4,10]. All Cina 200C- and Aakashmoni 200C- treated groups were received 10ml/plant test solutions (7.2 mg Cina 200C- and Aakashmoni- globules/ml concentration) respectively. The infected untreated with Cina- and Aakashmoni- (control) groups were similarly received 10 ml/plant control solutions (7.2mg- 90% ethanol soaked globules/ml concentration) [2-4,10]. It is noted that the infected untreated with Cina- and Aakashmoni (controls), were not untreated, but treated with the solution made from sugar pills soaked in the alcohol medium. The infected untreated (controls) were only treated with the solutions made from sugar globules in the alcohol medium (i.e. without medicine Cina - and Aakashmoni). At

fifteen days after the second treatment all the parameters of diseases were assessed again for each group [2-4,10]. All the data were used for statistical analysis by student's t-test.

### Analysis of residue

A thin layer chromatography plate (TLC) was made with silica gel [34]. Mulberry leaves, collected one day after last treatment were homogenized in a blender and extracted with ethanol. The residue was applied at one end of the plate as a small circular spot. The initial spot should be compact for reproducible  $R_f$  values and zones should always be placed at the same distance from the surface of developer [34]. Here, the residues run in thin layer chromatography plate (TLC) with the standard from the Cina 200C- and Aakashmoni 200C- test substances [10, 34].

### Rearing of silkworms

The eggs of a mother moth of the multivoltine 'Nistari' race (*Bombyx mori* L.) supplied by Regional Sericultural Research and Training Institute, Berhampore-742101, India, after hatching (93% hatching rate) and brushing 1<sup>st</sup> stage silk worm larvae in the rearing tray, the larvae were divided into three batches (180 silkworm larvae/batch) and reared [2-4,7-9,35]. The larvae of infected untreated batch (control) were fed with the leaves of pathogen infected diseased leaves of mulberry plants from infected untreated (control) plots and the larvae of infected treated two-batches were fed with the leaves of Cina 200C- and Aakashmoni 200C -treated leaves of mulberry plants from infected treated respectively. Fresh leaves were given to the larvae 4-times daily. Mulberry leaves were used for feeding fifteen days after the last treatment with both the drugs. The larvae were kept inside the rearing chamber at  $27 \pm 2^\circ\text{C}$  and  $70 \pm 15\%$  RH. The fresh weight of the larvae and that of the leaves served were recorded daily for each batch until the larvae started spinning. The consumption of fresh leaves [(Fresh leaves served – Dry leaves residues - Fresh leaves initially consumed) X Moisture loss], number of feeding and number of feeding day to cocoon formation, number of escaping feeding during moulting, moulting span days and mortality rate were recorded. The fresh silk gland weight of mature 5<sup>th</sup> instar larvae (before start spinning), starting time to spinning, span of spinning, fresh cocoon weight, fresh shell weight, silk layer ratio (SR% = Shell weight/Cocoon weight $\times 100$ ), effective rate of rearing (ERR % = Number of cocoon harvested/

Number of silk worm hatched  $\times 100$ ), sex ratio percentage (Number of male adult emerged/Number of female adult emerged $\times 100$ ) and egg laying capacity of mother moth were determined [2-4,7-9,35]. For statistical analysis by student's t- test, ten mature 5<sup>th</sup> instar silkworm larvae for fresh silk gland weight and ten cocoons for fresh shell weight were dissected out in each batches including replica of all batches [2-4,7-10,35]. All the data from rearing trial were used for statistical analysis by student's t- test.

## Results

### Estimation of the nematode population from field trial

The initial nematode populations [*Meloidogyne incognita* (Kofoid & White) Chitwood], stretching over an area of 5.6acre of mulberry plantation, were  $1779 \pm 43 J_2$  per 200g of soil and  $830 \pm 45 J_2$  per 2g of root. The nematode populations in the demarcated 0.16 acre, were  $1950 \pm 11 J_2$  per 200g of soil and  $615 \pm 15 J_2$  per 2g of root [before treatment (Day-0)].

### Mortality test

It was observed that Cina 200C- and Aakashmoni 200C- had no toxic effects on nematodes mortality within the exposure period of 12 hours at room temperature ( $25 \pm 2^\circ\text{C}$ ). For this reason, no data were presented in the results section.

### Analysis of residue

There had left no toxic residues of Cina 200C- and Aakashmoni 200C- in all the infected -treated plants by thin layer chromatography plate (TLC). For this reason, no data were presented in the results section.

### Root-knot disease

Table 1 shows the effects of Cina 200C- and Aakashmoni 200C- on *Meloidogyne incognita* pathogens infected mulberry plants in a field trial replicated thrice ( $P < 0.01$  by 't'- test). All naturally infected plants (treated plant group) treated with Cina 200C- and Aakashmoni 200C- showed increase number and surface area of leaves, and higher protein content in leaves and root than infected untreated (control) plants (untreated plant group). In all infected Cina 200C- and Aakashmoni 200C- treated plants, the population of root-knot nematodes decreased

Treatment batches (20plants / plots & 8 plots/batches)*	Average No. of leaves/plant*		Average Surface area of leaves (sq.cm)*		Average Protein content (%)+				Average Nematode population +				Average No. of rootgalls/plant +	
	Day-0	Day-30	Day-0	Day-30	Leaf		Root		Soil (200g)		Root (2g)		Day-0	Day-30
					Day-0	Day-30	Day-0	Day-30	Day-0	Day-30	Day-0	Day-30		
Infected Untreated (Control)	380ax $\pm 12.67$	430by $\pm 13.43$	7885ax $\pm 157.70$	24516by $\pm 408.60$	2.98ax $\pm 0.13$	6.75by $\pm 0.25$	4.38ax $\pm 0.16$	7.82by $\pm 0.30$	1937ax $\pm 74.50$	78by $\pm 3.39$	639ax $\pm 24.57$	107by $\pm 5.09$	1197ax $\pm 46.03$	221by $\pm 8.50$
Infected Cina 200C- treated	382ax $\pm 12.83$	434by $\pm 10.12$	7883ax $\pm 143.30$	25217dy $\pm 387.91$	2.99ax $\pm 0.12$	6.78by $\pm 0.24$	4.38ax $\pm 0.15$	7.88cy $\pm 0.26$	1935ax $\pm 74.30$	66cy $\pm 2.35$	639ax $\pm 22.03$	55dy $\pm 2.39$	1207ax $\pm 46.42$	187cy $\pm 6.67$
Infected Aakashmoni 200C- treated	380ax $\pm 12.83$	436by $12.12 \pm$	7882ax $\pm 143.30$	25215dy $\pm 327.02$	2.99ax $\pm 0.8$	6.78by $\pm 0.12$	4.38ax $\pm 0.15$	7.89cy $\pm 0.22$	1933ax $\pm 74.34$	62cy $\pm 2.32$	639ax $\pm 22.03$	54dy $\pm 2.32$	1208ax $\pm 40.42$	184cy $\pm 6.64$

\*- Means average values of 40 plants in triplicate.

+-. Means average values of 20 samples in triplicate.

'Day-0'- Means before treatment.

'Day-30'- Means after treatment.

'a,b'- Significant difference by t-test ( $P < 0.01$ ) in the same column.

'x,y'- Significant difference by t-test ( $P < 0.01$ ) in the same row between day-0 and day-30 of each character.

**Table 1:** Effects of Cina 200C- and Aakashmoni 200C- on *Meloidogyne incognita* infected mulberry plants in a field trial.

significantly in rhizospheric soil and as well as in roots than infected untreated (control) plants. The number of root galls also decreased significantly after Cina 200C- and Aakashmoni 200C- treatment.

### Foliar diseases

Table 2 shows only the effects of Aakashmoni200C on leaf spot, powdery mildew, mosaic viral and tukra diseases of mulberry plants in a field trial replicated thrice assessed initially (Day- 0) and after a period of 30 days (Day- 30) by 't'- test (P<0.01). Aakashmoni 200C significantly reduced the number of leaves infected with leaf spot, powdery mildew, mosaic viral and tukra as compared to the pre-treatment condition (Day- 0). The percentage of control achieved were 62.08 for leaf spot, 77.89 for powdery mildew, 64.91 for mosaic virus and 38.42 for tukra infection as compared to the pre-treatment level (Day- 0). In case of infected untreated plots leaf spot, powdery mildew, mosaic viral and tukra diseases showed naturally 27.80%, 17.76%, 29.37% and 21.20% reduction respectively, in 30 days (Day -30).

### Effects on feeding silkworms

Table 3 shows the effects of Cina 200C- and Aakashmoni 200C- on diseased infected mulberry plants in a silkworm rearing and field trial replicated thrice on the feeding, growth and mortality of silkworms (P<0.01 by 't'-test). The average consumption of leaves by the 5<sup>th</sup> instars, average number of feeding to cocoon formation, average number of feeding day to cocoon formation, average number of escaping- feeding during moulting and average moulting span days were less for Cina

200C- and Aakashmoni 200C- treated plants than for infected untreated (control) ones. The average mortality rate (%) was nil with Cina 200C- and Aakashmoni 200C- treated plants groups and 56% with infected untreated (control) one. However, the average fresh weight of the 5<sup>th</sup> instars larvae were higher with Cina 200C- and Aakashmoni 200C- treated plants than with infected untreated (control) one.

### Effects on silk production and rearing practices

Table 4 shows the effects of feeding Cina 200C- and Aakashmoni 200C- treated mulberry leaves on silk production, spinning characters and rearing practices in a silkworm rearing and field trial replicated thrice (P<0.01 by 't'-test). The average fresh silk gland weight, average fresh cocoon weight, average fresh shell weight and average shell ratio (SR%) were higher with Cina 200C- and Aakashmoni200C - treated plants than with infected untreated (control) one. It is notable that average starting time to spinning day and average span of spinning day (i.e. duration of span) were fewer with the Cina 200C- and Aakashmoni 200C - treated plants than with infected untreated (control) ones. Average effective rate of rearing (ERR %), average sex ratio percentage and average egg laying capacity were significantly higher with all Cina 200C- and Aakashmoni 200C- treated groups.

### Discussion

The homeopathic drugs; Cina 200C- and Aakashmoni 200C- ones again confirm that the cost effective drugs not only reduced root-knot, leaf spot, powdery mildew, viral and tukra diseases but also improved

Treatment groups (20plants/ Plot & 8 plots/ group)	Average number of disease-infected leaves / plant ( % )							
	Leaf spot		Powdery mildew		Mosaic		Tukra	
	Day-0	Day-30	Day-0	Day-30	Day-0	Day-30	Day-0	Day-30
Infected Untreated (Control)	70.58ax ± 2.28	98.38ay ± 3.93 (<27.80%)	80.75ax ± 3.23	98.51ay ± 3.94 (<17.76%)	68.68ax ± 2.74	98.05ay ± 4.10 (<29.37%)	57.15ax ± 2.38	78.35ay ± 3.26 (<21.20%)
Infected Aakashmoni 200C -treated	70.53ax ± 2.71	8.45by ± 2.71 (>62.08%)	80.86ax ± 3.11	2.97by ± 0.01 (>77.89%)	68.32ax ± 2.62	3.41by ± 0.13 (>64.91%)	57.11ax ± 2.37	18.69by ± 0.81 (>38.42%)

Day-0 Means before treatment. Day-30 means after treatment.

a, b - Significant difference by 't'-test (P<0.01) in the same column.

x, y - Significant difference by 't'- test (P<0.01) in the same row between day-0 and day-30 of each character.

( ) - Figures in the parentheses show percentage of reduction on day-30 as compared to the initial level on day-0 in the same row.

**Table 2:** Effects of Aakashmoni 200C- on leaf spot, powdery mildew, mosaic and tukra diseases of mulberry plants in a field replicated thrice assessed initially (Day-0) and after a period of 30 days (Day-30).

Treatment batches (180 larvae/ batch)*	Average number of						
	Consumption of leaves(g) (5 <sup>th</sup> instar)*	Feeding to cocoon formation*	Feeding- day to cocoon formation*	Escaping feeding during moulting*	Moulting span day (1 <sup>st</sup> to 5 <sup>th</sup> instar)*	Larval fresh weight (g) (5 <sup>th</sup> instar)*+	Mortality rate (%)*
Infected Untreated (Control)	4.03a ± 0.15	76.00a ± 2.37	19.00a ± 0.50	51.00a ± 1.75	13.00a ± 0.39	1.48a ± 0.03	56.00 ± 2.43
Infected Cina 200C -treated	2.46b ± 0.09	62.00b ± 1.93	15.00b ± 0.44	20.00b ± 0.68	5.00b ± 0.15	2.63b ± 0.06	Nil
Infected Aakashmoni 200C -treated	2.42b ± 0.04	60.00b ± 1.92	15.00b ± 0.40	20.00b ± 0.62	5.00b ± 0.13	2.61b ± 0.05	Nil

a, b - Different small letters in a column show significant difference by 't'- test (P<0.01).

\* - Average values of 180 silk worm larvae in triplicate.

+ - Average values of 10 silk worm larvae were dissected in triplicate.

**Table 3:** Effects of disease-infected and Cina 200C- and Aakashmoni 200C - treated mulberry plants in a field on the feeding and growth of silkworms in the silkworms rearing trials (replicated thrice).

Treatment batches (180 larvae/ batch)*	Average								
	Silk gland fresh weight(g) (5 <sup>th</sup> instar)+	Starting time to spinning (at day-)*	Span of spinning day*	Cocoon fresh weight (g)*	Shell fresh weight (g)+	Shell ratio (SR %)+	Effective rate of rearing (ERR %)*	Sex ratio (Male / Female %)	Egg laying capacity
Infected Untreated (Control)	0.98a ± 0.03	34.00a ± 1.30	10.00a ± 0.45	0.85a ± 0.03	0.11a ± 0.01	12.94a ± 0.49	21.37a ± 0.63	76.00a ± 1.94	320.00a ± 13.91
Infected Cina 200C-treated	1.98b ± 0.07	20.00b ± 0.51	3.00b ± 0.09	1.09b ± 0.04	0.24b ± 0.02	22.01b ± 0.67	97.43b ± 2.16	68.00b ± 1.74	540.00b ± 11.73
Infected Aakashmoni 200C-treated	1.98b ± 0.04	20.00b ± 0.42	3.00b ± 0.06	1.09b ± 0.02	0.24b ± 0.01	22.01b ± 0.42	97.48b ± 2.16	68.00b ± 1.72	540.00b ± 11.71

a,b - Different small letters in a column show significant difference by 't'- test (P<0.01).

\* - Average values of 180 silk worm larvae in triplicate.

+ - Average values of 10 silk worm larvae and cocoon were dissected in triplicate.

**Table 4:** Effects of disease-infected and Cina 200C- and Aakashmoni 200C - treated mulberry plants in a field on the growth of silk gland, spinning time, cocoon, shell, rearing, sex ratio and egg laying capacity in the silkworms rearing trials (Replicated thrice).

the nutritive value of the treated leaves of infected plants [10].

- From this field trial, we confirm that Cina 200C and Aakashmoni 200C also improves the nutritive value of the treated leaves [2,6-10] which directly influences on the consumption of leaves, number of feeding and number of feeding day to cocoon formation, and indirectly affects on moulting stage in all the Cina 200C- and Aakashmoni 200C- treated groups from these trials.
- And due to ill development of infected untreated (control) batches larvae took more time to moult which is proved from the number of escaping feeding during moulting [2,6-10].
- Higher nutritive value of treated plants contribute to higher growth of silkworm larvae, silk gland weight, cocoon weight and shell weight which increase silk production significantly [2,6-10] for commercial purpose [10].
- The improved health of the larvae, cocoon weight, silk gland and shell weight from the Cina 200C- and Aakashmoni 200C-treated groups of the infected plants might have resulted in the fewer starting time to spinning and span of spinning day and the total elimination of the mortality rate [2,6-10]. However, Cina 200C- and Aakashmoni 200C is too dilute to contain drug molecules [2,6-10,36]. Naturally, the drug might not have affected the nematode directly [2,6-10] and for this reason, no mortality occurs.
- The effective rate of rearing (ERR %) is very high in all Cina 200C- and Aakashmoni 200C- treated treatment batches which enriches the sericulture industry in many ways, specially for commercial purpose [2,6-10].
- The mulberry leaves did not contain any toxic residues of the Cina 200C- and Aakashmoni 200C- test substances by the thin layer chromatography (TLC). It is reported that Cina- and Aakashmoni- at ultra high dilution has physical basis in the form of charge transfer interaction and altered rate of tumbling in the specific part of the molecules of the diluents medium [2,6-10,36].
- Rather, the drug Cina 200C- and Aakashmoni 200C- might have induced natural defense response in the test plants against nematode parasites and has conferred defense response on growing larvae [2,6-10,36].

- In fact, it is surprising that all infected Cina 200C- and Aakashmoni 200C- treated plants not only are less affected by nematodes but also have a better growth than the infected untreated with Cina 200C- and Aakashmoni 200C (control) plants [2,6-10,36].
- And the positive effects of growth may be responsible for defense resistance against pathogens [2,6-10,36,37]. Both the drugs also improve the plant growth effectively which directly increase photosynthesis rate and significantly reduce CO<sub>2</sub> in the environment. So we can say that Cina 200C- and Aakashmoni 200C might have induced synthesis of many new proteins which have stimulated increased photosynthesis rate, stomatal activity and water retention capacity of Cina 200C- and Aakashmoni 200C- treated plants [2,6-10,36,37,38].
- The positive effects of growth on infected Cina 200C- and Aakashmoni 200C- treated plants may not only be responsible for defense resistance to nematodes pathogen but also improves growth of silkworm larvae and silk gland weight, cocoon weight, shell weight and effective rate of rearing (ERR %) [2,6-10,36,37,38] which increase silk production for commercial purpose. It is proved from the result that silk production is higher in the Cina 200C- and Aakashmoni 200C-treated groups than infected untreated with Cina 200C- and Aakashmoni 200C- (control) groups.

## Conclusion

These results once again suggest that plant diseases (like nematodes, fungus, virus, bacteria and insects etc) might be effectively controlled by the potentized cost effective homeopathic medicines Cina 200C- and Aakashmoni 200C- at an extremely low dose and also increases silk production and effective rate of rearing commercially which directly enriches sericulture industry as well as agriculture sector. Both the potentized-biopesticide-homeopathy-drugs also improve the plant growth effectively which directly increase photosynthesis rate and significantly reduce CO<sub>2</sub> in the environment. These cost-effective homeopathic medicines are easily available, biodegradable, non-phytotoxic and non-pollutant as well as conserve our biodiversity which will contribute towards "Sustainable Environment, Health and Development".

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