



## Enriched Science and Technology Communication Economy in Agriculture by Use of Acacia sides as Potential Bio-Agents against Various Pathogens

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### Abstract

Plant diseases, caused by different pathogens like nematodes, fungus, virus, bacteria and insects etc., infest almost all kinds of cash and vegetables crops affecting the economic value and agri-market of the world. Mulberry is economical plants because silk production depends on the nutritive quality of the leaves which is hampered by different pathogens attack. Pesticides are the most effective means of control, but they are expensive and as well as environmentally unfriendly also. To move forward, it will require new and more efficient solutions, technologies and products. Present investigation have revealed that Acacia sides (A&B) are highly effective at a dose of 1 mg / mulberry plant (*Morus alba* L., cv. S1) in ameliorating diseases caused by plant pathogens; Meloidogyne incognita root-knot nematodes causing root-knot disease, Cercosporammoricola fungus causing leaf spot disease, Phyllactiniacorylea fungus causing powdery mildew disease, mosaic virus causing mosaic disease and Maconellicoccushirsutus mealy bug causing tukra disease. Acacia sides were highly effective in ameliorating mulberry diseases and were increased the protein content of mulberry leaves. Silkworms larvae feeding on the leaves of treated plants showed improved growth of silkworms, increased cocoon weight and shell weight, fewer feeding to cocoon formation, zero mortality rate and increased the effective rate of rearing, sex ratio percentage and egg laying capacity of mother moth. The results, showed that Acaciasides (A&B) use as “Potential Eco-friendly Bio-agents Against Various Pathogens of Plants Enriching Science and Technology Communication Economy in Agriculture Significantly without

Disturbing Biosphere”. It also conserve our biodiversity which will contribute towards “Sustainable Climate, Health and Development” by controlling plant diseases which is sometime devastating to all kinds of -natural and -artificial vegetation and may also provide a unique platform for showcasing the research across the globe and progress the further advanced research in ‘Agriculture, Horticulture and Entomology’ deals with economy. In near future, a clinical study may be arranged for discovery of ‘Vaccine’ by using Saponins from Boi-Agent Acaciasides which may kill the novel Corona virus COVID-19 by boosting our immune system.

**Keywords:** Acacia sides; Agriculture; Bio-agents; Pathogens; Mulberry; Silkworms; Science and Technology Communication Economy; Vaccine

### Introduction

Mulberry (*Morus alba* L.) is an important economical crop plants 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 [1-10]. 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, 9-19]. 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 [1-21]. 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. The lack of regular and systematic studies on the occurrence of various diseases and epidemic is responsible for the recurring loss in leaf yield [1-21].

## Characteristic Features of the Diseases

### Root-Knot Disease

Root-knot nematode, *Meloidogyne incognita* (Kofoid & White) Chitwood, infesting mulberry forming root galls has been reported from all countries and it is serious due to perennial nature of mulberry and endo-parasitic habit of the nematode. The disease reduces 10-12% leaf yield in addition to affecting the leaf quality for silkworms feeding [21-26].

### Leaf spots disease

*Cercosporamoricola* (Cooke) fungus, causing main leaf spot disease, is the most devastating throughout the country and it causes 10-35 % loss in leaf yield reducing moisture, proteins adversely and ultimately the quality and quantity of cocoons. It produces minor circular light brown spots on the leaves which gradually increase in size and turn dark brown [1-11].

### Powdery Mildew Disease

*Phyllactiniacorylea* (Pers.) Karst fungus, causing powdery mildew disease, is the most common and wide spread economically important disease reducing 10-30 % leaf yield. Biochemically these leaves are poor nutritive value showing reduction in moisture, protein and sugar. Feeding of diseased leaves affect the growth and development of silkworms. Symptoms of disease can easily identified by the appearance of white patches on the lower surface of the leaves. As the disease advances the patches spread to entire leaf surface and turn to blackish colour and become coarser and leathery, reducing the crude protein content by as much as 33 % [1-26].

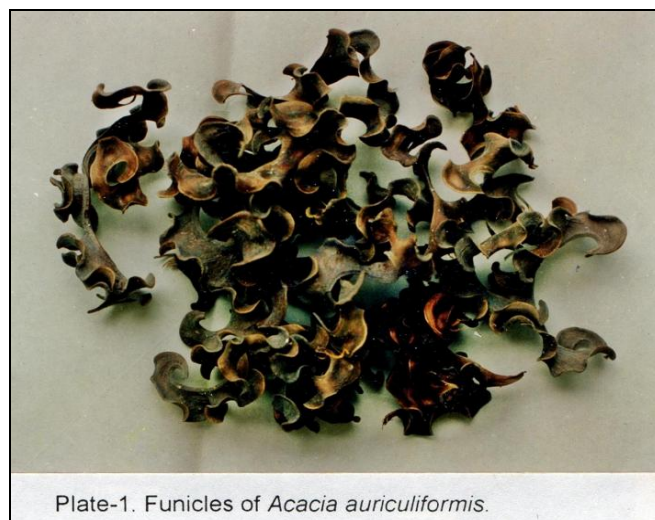
### Mosaic Disease

The symptoms of mosaic disease, caused by mosaic virus, are inward curling of leaves, particularly leaf margin and tip with chlorotic lesions on the leaf surface, stunted growth and suppressed leaf size [1-26].

### Tukra Disease

*Maconellicoccushirsutus* (Green) (Pseudococcidae) is commonly known as mealy bug and is associated with mulberry plants showing symptoms popularly known as 'Tukra disease'. The leaf yield is tremendously reduced the leaves have depleted in nutritive value. The affected apical

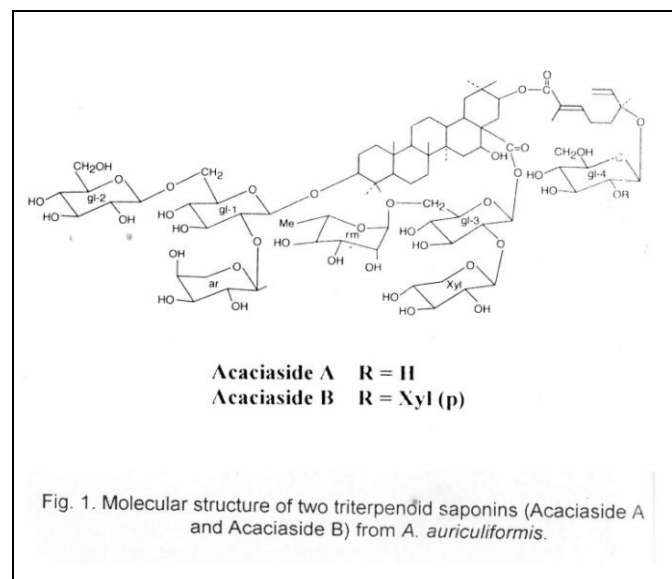
shoot shows retarded growth. Flattening of the apical shoot and wrinkling of the affected leaves are also associated. The leaves become dark green colour. The disease reduces plant growth, leaf yield and leaf protein content significantly [1-26]. Traditional chemical control using chemical compounds available for few decades is in declining status internationally [1-27]. Thus, there remains a need for developing effective biocompounds, which would be cheap, non-phytotoxic and non-pollutant. Plants offer safe and effective substitutes for chemical control against pathogens [10-16, 27-29]. It has been already observed that the extract from the funicles of *Acacia auriculiformis* A. Cunn. (Plate 1) is effective in reducing mulberry diseases leaving no residual toxicity in the leaves to affect the growing silkworm larvae [10-19, 27-29]. It has also been isolated the pure compounds acaciasides (A&B) from the crude extract of the funicles of *Acacia auriculiformis* A. Cunn. [9-12, 24-31].



The purpose of the present investigation is to further confirm the efficacy of the acaciasides at very low dose, use as bio-agents isolated from the crude extract of acacia funicles, in ameliorating root-knot disease of mulberry (*Morus alba* L., cv. S1) caused by *Meloidogyne incognita* (Kofoid & White) Chitwood root-knot nematodes pathogens and also to find out if the pure compounds acacia sides (A&B) can reduce the four foliar diseases, caused by pathogens, under field condition. The foliar diseases were: leaf spot disease caused by *Cercosporamoricola* (Cooke) fungus pathogens, powdery mildew disease caused by *Phyllactiniacorylea* (Pers.) Karst fungus pathogens, mosaic disease caused by mosaic virus pathogens and tukra disease caused by *Maconellicoccushirsutus* (Green) mealy bug pathogens. The effects of the leaves of the acaciasides- treated plants on the leaf consumption, growth of silkworm's larvae, silk gland weight and effective rate of rearing (ERR) were also observed.

Bio-agents (any biological agents of biological origin derived from either plants or animals source) provide a new class of biological compounds which stand as a suitable and useful alternative to conventional but hazardous methods of chemical control [11]. In course of our experiments with anti-nematode agents, acaciasides (A&B), it was observed that the

mulberry plants (*Morus alba* L., cv. S1) 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 acaciasides (**Figure 1**). The result would be more realistic in terms of the potentiality of the Acaciasides (A&B), use as “Eco-friendly Bio-Agents against Various Pathogens of Plants Enriching Science and Technology Communication Economy in Agriculture” is a question as a Phytologist or a general Plant Biologist or Entomologist or Economist.



## Material 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 % [11-12].

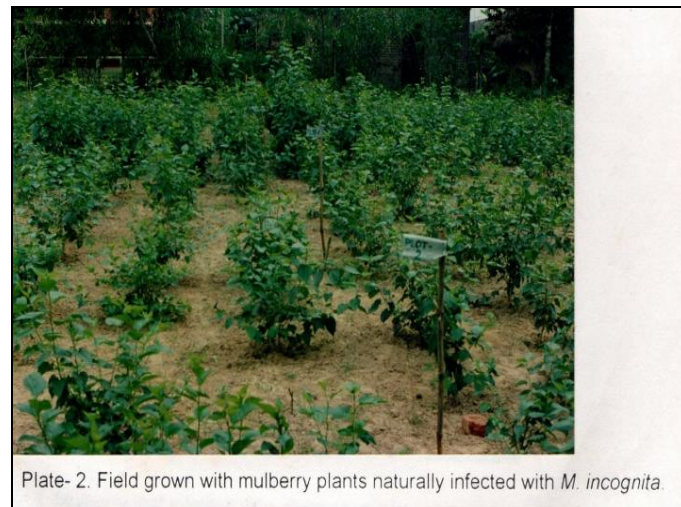
### Estimation of the Nematode Pathogen Population

Soil and root samples [11-12, 32-33] 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, two areas (in the same locality and climatic condition) each measuring 0.02 ha; one naturally rootknot disease infected untreated field and other naturally root-knot disease infected treated field, were demarcated in the mulberry field where there were no soil differences as well as environmental factor.

### Preparation of Fields

The first 0.02 ha nematode infected (2863 + 55 J2 / 1 kg of soil) sandy soil area (18889.76 x 1066.80 x 45.72 cm<sup>3</sup>) 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 [7-8, 11-12, 14-15, 17, 24-26, 31-35] and this naturally infected soil-filled area, demarking untreated field (**Plate-2**), was replicated thrice.



The other 0.02 ha (18889.76 x 1066.80 x 45.72 cm<sup>3</sup>) 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. This naturally infected soil-filled area, demarking treated field (**Plate-2**), was also replicated thrice.

### Plantation of Mulberry Cutting

Mature three years old mulberry cutting, *Morus alba* L., cv. S1 (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 [12-16, 32-33, 36]. At least 80 number at random rhizospheric soil sampling (200g in each sample) were collected from rhizospheric root-soil area of root (10-15cm X 10-15cm) and at least 40 number at random root sampling (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.

### Division of Groups and Plots

After three months growth of mulberry, *M. incognita* population were estimated in the rhizospheric soil as well as roots [12-19, 32-33, 36] (at least 40 at random sampling in

each area) of mulberry plants in each areas of mulberry field. The *M. incognita* infected mulberry plants were achieved growth of 50-60 cm in height. The infected mulberry plants were divided in to 16 plots, each measuring the area of 472.44cm X 533.4cm X 45.72cm. The mulberry plants divided into two plant groups; untreated plants group and treated plant group and each group has 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, 37]. 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 [12-19, 23-26, 31-38] of infected mulberry plants. Total number and surface area of leaves of all plant groups were counted [11-19, 23-26, and 31-38]. Total number of root-galls/plant were counted in the infected roots of mulberry plants [11, 19, 33-36, 40]. The total protein content of the leaf and root samples (10 at random sampling / plot) from each of the 16 plots was determined [36, 40-41]. 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 [4-18, 19, 23, 28-29, 33].

### Foliar Diseases

The main foliar diseases, observed in the sericulture field, were: leaf spot disease caused by *Cercosporammoricola* (Cooke) fungus pathogens, powdery mildew disease caused by *Phyllactiniacorylea* (Pers.) Karst fungus pathogens, mosaic disease caused by mosaic virus pathogens and tukra disease caused by *Maconellicoccus* (Green) mealy bug pathogens. All the disease identified according to their characteristic symptoms by the experts concerned [1, 9-16, 20]. Diseased leaves of each type were counted in each plots [1, 11, 20, 23, 31, 34-35].

The percentage of disease infection based on diseased leaf surface area [9-16, 37, 42-44]. Preparation of crude *Acacia auriculiformis* extracts Air-dried and powdered funicles of *Acacia auriculiformis* A. Cunn. Were extracted with 90% ethanol at room temperature (25 + 2°C) for 15 days and were filtered for collecting extract. Later, the ethanol from the extract was removed by evaporation at room temperature (25 + 2°C). The residue, obtained after removal of the solvent under reduced pressure, was dried in a desicator over anhydrous calcium chloride [11, 19, 23, 26, 30, 34].

## Isolation of Acaciasides

Crude residue of *Acacia auriculiformis*- extracts from the funicles of *Acacia uriculiformis* A. Cunn. Were again successively extracted with petrol (60-80° C) and 90% ethanol. The ethanol extract, on removal of the solvent under reduced pressure, yielded a viscous dark brown mass. The extract was chromatographed on silica gel with petrol, petrol-chloroform (1:1), chloroform, chloroform-methanol (9:1, 7:3, 3:2, 1:1 and 2:3) as successive eluents. The chloroform-methanol (7:3 and 3:2) eluates were then combined fraction was found to be composed mainly of two compounds which were separated by repeated preparative HPLC employing Spherisorb S-10-ODS reversed phase column with the solvent system methanol water (7:3) at a flow rate of 4 ml / min and refractive index detector as white amorphous solids.

These two solid compounds, designated as acaciaside A and acaciaside B (Figure 1) according to their increasing order of polarity, were found to be triterpenoidsaponins by LiebermannBurchard, molisch and forth tests [11, 19, 23, 26, 30, 34].

### Mortality Test

Ten sets of cavity block with 1ml sterile tap water containing 50 larvae (J2) of *Meloidogyne incognita* were taken; five set was treated as control and other five were treated as treatment set. The acaciasides (A&B) were dissolved in sterile tap water at 0.0125 mg / ml forming acaciasides-test solution. To assess the direct effect of acaciasides- test solution, the water was removed by pipette and in all the treatment sets, immediately replaced by 1ml of test solutions (0.0125 mg acaciasides / ml concentration) were added, except the control and observed with every one hour interval for a period of 24 hours exposure period at room temperature (25±2°C). Immediately after observation of each block, nematodes were transferred to sterile tap water again to see if any recovery occurred after 4 hours [4-18, 45]. This mortality test was replicated five times.

### Preparation of Acaciasides-Test Solution

In our experiment, the mixture of acaciaside A and acaciaside B (3:2) was dissolved in sterile tap water at 0.0125 mg / ml forming acaciasides-test solution and used for treatment plots [12-13, 19].

### Treatment

Seventy six days after pruning, of mulberry plants, all the plots (treated groups and untreated groups) were done by foliar spray and soil drench @ 20ml / plant in each type of treatment (0.0125 mg acaciasides / ml concentration in case of treated groups) twice at an interval of 15 days with acaciasides- test solution and sterile tap- water respectively. Treatments were given in such a way that all the leaves and rhizospheric soil of the plants were completely drenched with test-solutions and tap-water. During spraying, the soil surface underneath each plant was covered with polyethylene sheet.

All the acaciasides- treated groups were received 80ml / plant test solutions (1 mg / plant) and other untreated- plant groups were similarly received 80 ml / plant sterile tap water respectively [12-13, 19]. It was told about untreated (control); these controls were only treated with the sterile tap water (i.e. without acaciasides-test solution). At fifteen days after the second treatment all the parameters of diseases were assessed again for each group [9-14, 17, 19]. All the data were used for statistical analysis by student's t-test.

### Analysis of Residue

Mulberry leaves, collected fifteen days after second treatment were homogenized in a blender and extracted with ethanol. The residue runs in thin layer chromatography plate (TLC) with the standard from the acaciasides- test substances. The test substances were acaciasides- test solution [9-15, 17, 19].

### Rearing of Silkworms

The eggs of a mother moth of the multivoltine 'Nistari' race (*Bombyxmori* L.) supplied by Regional Sericultural Research and Training Institute, Berhampore-742101, India, after hatching (93 % hatching rate) and brushing 1st stage silk worm larvae in the rearing tray, the larvae were divided into two batches (180 silkworm larvae / batch) and reared [9-15,17-18, 46]. The larvae of infected untreated batch (control) were fed with the leaves of pathogens infected diseased leaves of mulberry plants from infected untreated (control) plots and the larvae of infected treated batch were fed with the leaves of acaciasides-treated leaves of mulberry plants from infected treated. Fresh leaves were given to the larvae 4- times daily. Mulberry leaves were used for feeding fifteen days after the last treatment with acaciasides. The larvae were kept inside the rearing chamber at  $27\pm 2^{\circ}\text{C}$  and  $70 + 15\%$  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  $\square$  (Fresh leaves served – Dry leaves residues - Fresh leaves initially consumed) X Moisture loss  $\square$ , 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 5th instar larvae (**Plate 6**) (before start spinning), starting time to spinning (**Plate 7**), span of spinning, fresh cocoon weight, fresh shell weight, silk layer ratio ( $\text{SR \%} = \text{Shell weight} / \text{Cocoon weight} \times 100$ ), effective rate of rearing ( $\text{ERR \%} = \text{Number of cocoon harvested} / \text{Number of silk worm hatched} \times 100$ ), sex ratio percentage ( $\text{Number of male adult emerged} / \text{Number of female adult emerged} \times 100$ ) and egg laying capacity of mother moth were determined [9-15, 17-18, 46].

For statistical analysis by student's t- test, ten mature 5th 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 [9-15, 17-18, 46]. All the data from rearing trial were used for statistical analysis by student's t- test.

### Science and Technology Communication Economy

The activity of students, researchers, teachers, staff, community, photographers, visitors, different scientist, administrators, institutions, farmers, NGO and media personnel, -campaign or -aware or -make news or -publication regarding the importance of "Acaciasides use as potential Bio-Agents against various plant pathogens: Enriching Science and Technology Communication Economy in Agriculture and Biodiversity Conservation issues" in different audio visual media (TV channels), social media, webpages, news papers and journals is recorded [9-13, 18-26, 47].

### Results

#### Mortality Test

It was observed that acaciasides (A&B) had direct toxic effects on nematodes mortality within the exposure period of 24 hours because all the nematodes died and any recovery of nematodes occurred after 4 hours and no mortality occurred in the control.

#### Analysis of Residues

Mulberry leaves collected fifteen days after the second treatment, did not contain any toxic residue of the acaciasides-test substance.

#### Root-Knot Disease

Table 1 shows the effects of acaciasides 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 acaciasides (A&B) 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 acaciasides- treated plants, the population of root-knot nematodes decreased significantly in rhizospheric soil and as well as in roots than infected untreated (control) plants. The number of root galls also decreased significantly after acaciasides- treatment.

Treatment groups (20 plants/ Plot & 8 plots / group )	Average number of leaves / plant		Average surface area of leaves (sq.cm)		Average protein content (%)				Average nematode population				Average number of root galls/plant	
					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	Day-0	Day-30	Day-0	Day-30
Infected Untreated	389ax	373ay	7998ax	8003ax	2.98ax	2.01ay	4.39ax	3.29ay	1931ax	2406ay	638ax	2084ay	1233ax	2078ay
(Control)	±15.71	±14.21	±179.64	±177.61	±0.12	±0.07	±0.17	±0.07	±77.10	±96.64	±24.22	±97.40	±49.13	±78.62
Infected Treated	387ax	438by	7992ax	25246by	2.98ax	8.96by	4.39ax	7.85by	1933ax	69by	639ax	49by	1229ax	129by
(Control)	±12.81	±12.12	±179.48	±387.96	±0.12	±0.29	±0.17	±0.02	±77.11	±2.33	±22.03	±1.35	±48.91	±3.27

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 acacia sides (A&B) on Meloidogyne incognita pathogens infected mulberry plants in a field trial replicated thrice.

### Foliar Diseases

Table 2 shows the effects of acaciasides 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). Acaciasides (A&B) significantly reduced the number of leaves infected with leaf spot, powdery mildew, mosaic viral and tukra (**Plate 3**) 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 (**Plate 4**), 64.91 for mosaic virus (**Plate 5**) 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).

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-30	Day-30	Day-0	Day-30
Infected Untreated (Control)	70.58ax	98.38ay	80.75ax	98.51ay	68.68ax	98.05ay	57.15ax	78.35ay
(Control)	±2.28	±3.93(<27.80%)	±3.23	±3.94(<17.76%)	±2.74	±4.10 (<29.37%)	±2.38	±3.26 (<21.20%)
Infected Treated	70.53ax	8.45by	80.86ax	2.97by	68.32ax	3.41by	57.11ax	18.69by
(Control)	±2.71	±2.71 (>62.08%)	±3.11	±0.01 (>77.89%)	±2.62	±0.13 (>64.91%)	±2.37	±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 acacia sides 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).



Plate- 3. Mulberry leaf infected with 'Tukra disease'.



Plate- 5. Mulberry leaf infected with 'Mosaic Virus'.

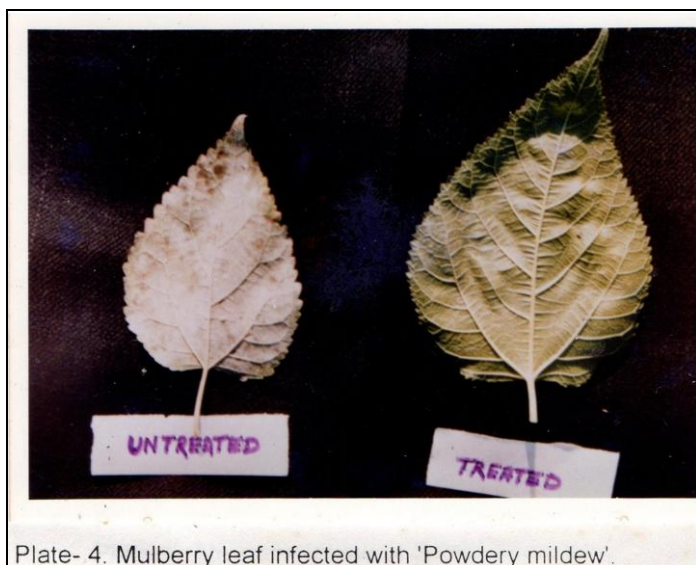


Plate- 4. Mulberry leaf infected with 'Powdery mildew'.

### Effects on Feeding Silkworms

Table 3 shows the effects of acaciasides 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 5th instars (Plate 6), average number of feeding to cocoon formation (Plate 7), average number of feeding day to cocoon formation, average number of escaping- feeding during moulting and average moulting span days were less for acaciasides (A&B)- treated plants than for infected untreated (control) ones. The average mortality rate (%) was nil with acaciasides- treated plants groups and 56% with infected untreated (control) one. However, the average fresh weight of the 5th instars larvae were higher with acacia sides- treated plants than with infected untreated (control) one.

Treatment batches (180 larvae/batch)*	Average number of						
	Consumption of leaves(g) (5th instar)*	Feeding to cocoon formation*	Feeding day to cocoon formation*	Escaping feeding during moulting*	Moulting span day (1st to 5th instar)*	Larval fresh weight (g) (5th instar)**	Mortality rate (%)*
Infected Untreated (Control)	4.03a	76.00a	19.00a	51.00a	13.00a	1.48a	56.00
	±0.15	±2.37	±0.50	±1.75	±0.39	±0.03	±2.43
Infected Treated	2.46b	62.00b	15.00b	20.00b	5.00b	2.63b	Nil
	±0.09	±1.93	±0.44	±0.68	±0.15	±0.06	

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 acaciasides- treated mulberry plants in a field on the feeding and growth of silkworms in the silkworms rearing trials (replicated thrice).



Plate- 6. 5th instar larvae of *Bombyx mori* L.

### Effects on Silk Production and Rearing Practices

Table 4 shows the effects of feeding acaciasides- treated mulberry leaves on silk production, spinning characters and rearing practices in a silkworm rearing (Plate 7) 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 acacia sides- 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 acaciasides- 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 acaciasides- treated groups.

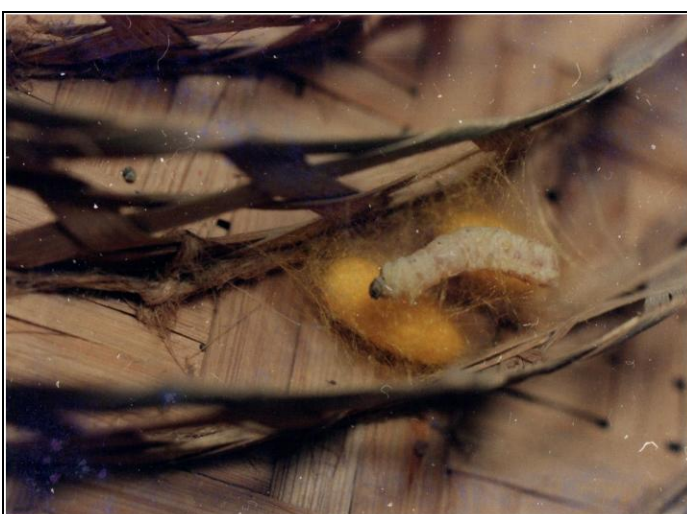


Plate- 7. Cocoon formation by 5th instar larvae.

Treatment batches (180 larvae/batch)*	Average number of								
	Silk gland fresh weight (g) (5th 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	34.00a	10.00a	0.85a	0.11a	12.94a	21.37a	76.00a	320.00a
	±0.03	±1.30	±0.45	±0.03	±0.01	±0.49	±0.63	±1.94	±13.91
Infected Treated	1.98b	20.00b	3.00b	1.09b	0.24b	22.01b	97.43b	68.00b	540.00b
	±0.07	±0.51	±0.09	±0.02	±0.01	±0.67	±2.16	±1.74	±11.73

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 acacia sides- 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).



### Here, Key Findings

- Here, acacia sides (A&B), isolated from the funicles of *Acacia auriculiformis* A. Cunn., were used against root-knot, leaf spot, powdery mildew, mosaic viral and tukra diseases caused by pathogens in a mulberry field trial. It was also observed the responses of silkworms feeding on mulberry leaves.
- Acacia sides were soluble in water and applied to 6 plots twice at an interval of 15 days @ 1 mg / plant infected with above mentioned pathogens by foliar spray and soil drench using 20ml solution for each type of treatment serving treatment plots. The remaining 6 plots treated with tap water serving control plots.
- Acacia sides were highly effective in ameliorating mulberry diseases and were increased the protein content of mulberry leaves. Silkworms larvae feeding on the leaves of treated plants showed improved growth, increased cocoon weight and shell weight, fewer feeding to cocoon formation, zero mortality rate and increased the effective rate of rearing.
- The results showed that acaciasides use as potential eco-friendly bio-agents against various pathogens of plants enriching agriculture significantly without disturbing biosphere.

### Science and Technology Communication Economy

The students, researchers, teachers, staff, community, photographers, visitors, different scientist, administrators, institutions, farmers, NGOs and media personnel campaign, aware, discussion, arrange workshops and seminars, make news and publish as abstract regarding the importance of “Acaciasides use as Potential Bio-Agents Against Various Plant Pathogens: Enriching Science and Technology Communication Economy in Agriculture and Biodiversity Conservation Issues” in different national- and local- audio visual media (TV channels), different social media, web pages, news papers and different -national and -international Journals as well as Congress Proceedings also. We are amazed for use of Acaciasides - directly or -indirectly help the society in various ways and may also provide a unique platform for showcasing the research across the globe and progress the further advanced research in Agriculture, Horticulture and Entomology deals with economy. Common people also realize the meaning of new and more efficient solutions, technologies, products and it has to improve Science and Technology Communication Economy forming joyful environment and fulfill its food and nutrition requirement which resist any kinds of chemical thresholds or natural infections for the climate change and resource productive economies enriching quality of environment.

### Discussion

The pure compounds acacia sides (A&B), isolated from the funicles of *Acacia auriculiformis* A. Cunn., Bio-Agents not only reduced root-knot, leaf spot, powdery mildew, viral and tukra diseases but also improved the nutritive value of the

treated leaves of infected plants [11-13, 19]. Acacia sides treatments 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 the infected treated groups from this trial. And due to ill development of infected untreated (control) batches silkworm larvae took more time to moult which is proved from the number of escaping feeding during moulting. Higher nutritive value of treated plants contributed to higher growth of silkworm larvae, silk gland weight, cocoon weight and shell weight which increase silk production significantly for commercial purpose [11-13, 19]. The improved health of the larvae, cocoon weight, silk gland and shell weight from the acacia sides- treated groups of 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. Or, the acacia sides might have infused in to mulberry leaves a substance that has conferred disease resistance on growing silkworm larvae by releasing defence-related natural products by plants [2-13, 17-18, 31, 34-35, 49]. The effective rate of rearing (ERR %) is very high in all acacia sides- treated treatment batches which enriches the sericulture industry in many ways, especially for commercial purpose. The mulberry leaves did not contain any toxic residues of the acacia sides- test substances by the thin layer chromatography (TLC). Rather, the acacia sides might have induced natural defense response in the test plants against all above mentioned pathogens and has conferred defense response on growing larvae [2-13, 17-19, 31, 34-35, 48-49, 52].

The present study clearly showed that acacia sides (A&B) were treated as effective or potential bio-agents and it had no direct toxic effect on plants but to the pathogens of mulberry plants. The bio-agents, acacia sides (A&B), could induced some resistance in mulberry against pathogens infection. It can be assumed that acacia side A and acaciaside B could induce synthesis of some antagonistic substance in the treated plants. Lectins accumulated in gall regions of root of *Hibiscus esculentus* infected with *M. incognita* [50]. Systemic acquired resistance can be induced by in different crop plants by localized virus infection, non-pathogenic and pathogenic microorganisms or their culture filtrates or by salicylic acid [2-18, 34, 51-54]. Plant-derived natural products have important functions in ecological interactions. In some cases these compounds are deployed to sites of pathogen challenged by vesicle-mediated trafficking. Polar vesicle trafficking of natural products, proteins and other, as yet uncharacterized, cargo is emerging as a common theme in investigations of diverse disease resistance mechanisms in plants [49]. Sequestration implies the involvement of specific transport process. For example, the defence-related triterpene glycoside avenacin A-1 is synthesized [55].

It is reported that a plant plasma membrane ATP binding cassette-type transporter is involved in antifungal terpenoid secretion [56]. Functional analysis has confirmed a role for this transporter in disease resistance [57]. Though, *M. incognita* is known to share common antigens with its host plants [58]. It appeared that during natural infection with the nematode, host plants showed minimal defense responses to

the nematode because of this antigenic similarity. Acacia sides (A&B) contain two triterpenoid saponins [12, 30, 34] and these saponins provide defence to the test plants against pathogens [59-63]. Acacia sides must be responsible for defense resistance of the mulberry. Acacia sides (A&B) may synthesis various antigens particularly (low molecular weight proteins) and induce defense responses involving a number of pathogenesis related protein in which the naturally infected plant pathogens fails to tolerate [12, 18, 31, 34, 59-63]. It is observed that in lady's finger plants treated with NE (nematode extract) showed the highest number of root proteins (no. 24) but in inoculated untreated root was 18 number and uninoculated untreated root was 11 number [12,18]. Those showed that NE served as a stimulus for the expression of many proteins particularly the defense – related proteins which later provide resistance to nematode infection. However, in the test plant were treated with NE after inoculation with live nematodes did not show much increase in number of proteins in root [23].

Those showed that nematode infestation somehow serve as a repressor for the expression of defense gene in plant [12, 18]. From this point of view, we must assume that acaciasides serve as a stimulus for the expression of many new induced defence-related PR-proteins by systemic acquired resistance which provides defense-resistance to various pathogens causing major diseases of plants. It can be told that acacia sides (A&B) acquiring systemic resistance could serve very effective eco-friendly bio-agents and promoted growth of test plants by inducing their defense responses of the host plants by expression of some new proteins against many plant pathogens infection causing major diseases and this bio-agents conserved our biodiversity and makes pollution free environment.

Now the key question is, whether plant-derived natural products (acaciasides A&B), can be used as potential Bio-Agents by inducing defence- response against various plant pathogens causing major mulberry diseases in a mulberry field trial and silkworms rearing. It is surprising that all infected acaciasides-treated plants not only are less affected by pathogens but also have a better growth than infected untreated (control) plants. The positive effects of growth may be responsible for defense resistance against pathogens. Acaciasides (A&B) might have induced synthesis of many new proteins which have stimulated increase photosynthesis rate, stomatal activity and water retention capacity of acacia sides- treated plants [2-9, 12-16, 64]. The positive effects of growth on disease-infected treated plants might not only be responsible for defense resistance to pathogens but also improved growth of silkworm larvae and silk gland weight, cocoon weight, shell weight, effective rate of rearing (ERR %), sex ratio percentage and egg laying capacity of mother moth with zero mortality rate were higher with all acacia sides- treated groups which increase silk production for commercial purpose. It is proved from the results that silk production is higher in all acaciasides- treated plants than infected untreated treated (control) plants. Now the answer is, bio-agents acaciasides was not only highly effective in

ameliorating mulberry diseases but also enriched sericulture industry as well as agriculture industry.

### Future Approach in Research

It is reported that the Bio-Agent acacia sides (A&B) is being used traditionally to overcome various medical complications like sore eyes, aches, rheumatism, allergy, itching, and rashes. Besides, it has also been proven for many pharmacological activities like central nervous system depressant activity, antioxidant, antimicrobial, antimalarial, anti-filarial, cestocidal, antimutagenic, chemopreventive, spermicidal, wound healing, hepatoprotective and antidiabetic activity and high efficacy. It has been used to treat several medical ailments due to its low toxicity and the presence of bioactive phytoconstituents [65]. Recently, in 'India Today' discuss regarding the "Trade in the time of COVID-19: The economic impact of corona virus on India and beyond" shows the estimates of India's aggressive 21-day lockdown could bring the country's growth down to 2.5 % from the 4.5 %. The effect of corona virus is likely to be seen long after medical science offers a cure or at least a vaccine [66, 67]. In a joint report from the World Health Organization (WHO) and the World Bank estimates the impact of such a pandemic at 2.2 per cent to 4.8 per cent of global GDP (US\$3 trillion) [66, 67]. In the Drug Target Review 2020, NovavaxInc, which contributed to the development of other epidemic vaccines, has announced it is currently in pre-clinical animal trials for several multiple nanoparticle COVID-19 vaccine candidates. The biotechnology company has announced its efforts to help in creating a vaccine against SARS-CoV-2. "Our previous experience working with other corona viruses, including both MERS and SARS, allowed us to mobilize quickly against COVID-19 and successfully complete the critical preliminary steps to engineer viable vaccine candidates," said Stanley Erck, President and Chief Executive Officer of Novavax which adjuvant is saponin-based and it has shown a "potent and well-tolerated effect" [68, 70]. It is already reported that the presence of chief constituents as flavonoids and two acylatedtriterpenoidbisglycosidesaponins present in Acaciasides (A&B) [3, 5, 11-12, 18, 30-31, 59, 66, 69, 71-75] (vide World Economic Forum's COVID Action Platform). So, in near future, a clinical study may be arranged for discovery of 'Vaccine' by using saponins from Bio-Agent Acaciasides which may kill the novel Corona virus COVID-19 by boosting human immune system and -generating superoxide anions, -initiating lipid peroxidation and -dissolving the fatty layer that coats of corona viruses.

### Conclusion

It can be concluded that plant diseases, like root-knot disease, leaf spot disease, powdery mildew disease, mosaic disease and tukra disease, might be effectively controlled by the cost effective acaciasides (A&B) by using as potential Bio-Agents against various plant pathogens in a mulberry field trial and silkworms rearing at an extremely low dose and commercially increases silk production which directly enriches sericulture industry as well as agriculture sector. And it would not only easily available, non-phytotoxic and non-

pollutant but also Acaciasides enrich “Science and Technology Communication Economy in Agriculture significantly without disturbing biosphere”.

It also conserve our biodiversity which will contribute towards “Sustainable Climate, Health and Development” by controlling plant diseases which is sometime devastating to all kinds of -natural and -artificial vegetation and may also provide a unique platform for showcasing the research across the globe and progress the further advanced research in Agriculture, Horticulture and Entomology deals with economy. And it would go a long way in tackling various pest of crops in a safe way by inducing their defense-responses of host plants against pathogens. In near future, a clinical study may be arranged for discovery of ‘Vaccine’ by using Saponins from Boi-Agent Acaciasides which may kill the novel Coronavirus COVID-19 by boosting our immune system and the whole world may retain in normal forms.

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