Pest infestation on the biochemical modulation of *Adhatoda vasica*

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ABSTRACT

Essential oil of *Adhatoda vasica* leaves contain vasicine, vasicinone and maiontone. The plant has lot of medicinal value against many diseases. Insecticidal activity of *Adhatoda vasica* was highlighted previously. Hence a thorough seasonal survey is taken up in *A vasica* to estimate the variation in the quantity and quality of the essential oils and alkaloids of *A. vasica* found in Virudhunagar District. The investigation proposes to study the various insect pests of the plants in exsitu cultivated plants of the Virudhunagar district. The data are compared with 50 plants collected from different areas and cultivated at the experimental gardens. Harvest was made at the interval of three months and a comparative analysis is presented after pest infestation. The above study is more valuable since the cost effectiveness of the tuberculosis treatment with *A. vasica* is commendable.

Key words : Pest occurrence, damaging potential and Phytochemical properties .

INTRODUCTION

Adhatoda vasica Nees (Acanthaceae) commonly known as vasaka distributed throughout India up to an altitude of 1300 m . The leaves, flowers, fruit and roots are extensively used for treating cold cough, whooping cough, chronic bronchitis and asthma, as sedative, expectorant and antispasmodic(Pandita, 1983). The study aims at making a qualitative and quantitative analysis of certain chemicals in *Adhatoda vasica*.

Antifeedent and Toxic activity Damaging Potential, Photosynthetic activities of *A. Vasica* is available. The study aims at making a qualitative and quantitative analysis of certain chemicals in *Adhatoda vasica*, to quantify the phytochemical variation in different seasons of the year, to establish the fact that there is annual pest rotation in *Adhatoda* altering the quality of active ingradieats and to study the damaging potential of the various insect pests in different seasons.

MATERIALS AND METHODS

Collection of plants

Three months old seedlings of *A. vasica* were collected from healthy and individual insect infested experimental garden at Virudhunagar .

Damaging potential

The study was carried out with four different pests infested *A. vasica* . The larva feeds the leaf alone leaving the midrib and vein regions. In the present study I to IV instar larvae were observed. The length and weight of the four instar larvae measured and tabulated . The consumed part of the leaf , unconsumed part of the leaf and total area of the leaf were measured graphically.

Twenty samples were analysed and the average value was considered. Developmental stages were analyzed with regard to the period in days and length and weight of codling moth larvae in every instar. Area consumed and area unconsumed in every larval instar was calculated and the ratio TA/CA was calculated and recorded (Singh *et al,* 1993).

Morphological Studies of Healthy Plant

Morphological studies were carried out in the healthy and pest infested leaves of *A. vasica*. The moisture content of healthy and infested leaves determined by the procedure of Mukharjee (2002) . The fluorescence analysis of healthy and infested leaves analysed by Kokate (1993) and crude fibre content determined by Wallis (1995).

The leaves were dried at a room temperature. Then the dried materials were converted into coarse powder by using a cutter mill and the fine powder was separated by passing through a mesh No : 60. The coarse powder was used for the pharmacognostical studies and phytochemical studies. Total ash, acid insoluble ash, water insoluble ash, sulphated ash, extractive value (Kokate, 1993), Chlorophyll (Welburn and Lichtenthaler , 1984), Sugar and Amino acid (Jayaraman, 1981), Protein (Lowry *et al.*, 1951), Phenol (Mahadevan, 1996), Tannin (Van Burden and

Robinson 1981), Flavonoid by Chromatographic method (Harbone *et al.*, 1984) , Anthocyanin (Mancinelli *et al.*, 1975) were determined.

RESULTS

Adhatoda vasica belongs to the family *Acanthaceae*. The other names are Adhatodai, Arusa, Adulsa, Bakas, Malabar nut tree. It is a small evergreen sub herbaceous bush . The plant *Adhatoda vasica* of Virudhunagar - Theni districts are usually infested by the larvae of codling moth, grasshopper and scale insects.

Adhatoda vasica was infested by grasshopper in the months of July to September. During this period the minimum rainfall recorded was 48.2 mm and maximum rain fall was 183.2 mm . The relative humidity ranged from 81.8 % to 84.7 % and the temperature ranged from 36.8ºC to 38.2ºC . Larva of codling moth infestation was maximum during the months of November to January . The rainfall recorded was 259.6 mm and the minimum rain fall was 34.8mm. The RH was 89.9 % to 92.4 % and the temperature varied from 32.1ºC to 33.4ºC . In the month of October - November scale insects infestation was recorded . During this period the rain fall varied from 255mm to 259.6mm .RH showed a range of 87.1 % to 92.4 % . The temperature was between 32.1ºC to 34.2ºC. Another pest weaver spider was noticed on the plant of *A. vasica* in the month of May and June . The rain fall ranged from 5.2 mm to 81.4 mm . The RH was 72.6 % to 75.3 % The temperature varied from 37.02ºC to 38.7ºC. Due to pest occurrence the damaging potential got modified and the percentage of consumption was 2.22 in nymph I stage 2.60 % in Nymph II stage , 5.06 % in Nymph III stage and 11.55 in Nymph IV stage . Adults showed almost negligible damage . There is a preference of small tender leaves of 49 cm^2 area for Nymph IV stage . Adults always preferred bigger leaves with 115.9 ± 7.428 cm^2 area for egg laying and surface feeding Length and Breadth values of healthy leaves showed 4.14 ± 0.181.The value decreased in pest infested leaves. The length and breadth of the healthy leaves showed 29.5 ± 0.983 , 7.45 ± 0.304. But the length /Breadth value decreased in pest infested leaves, excepting scale insect infested leaves which were comparable to the control.

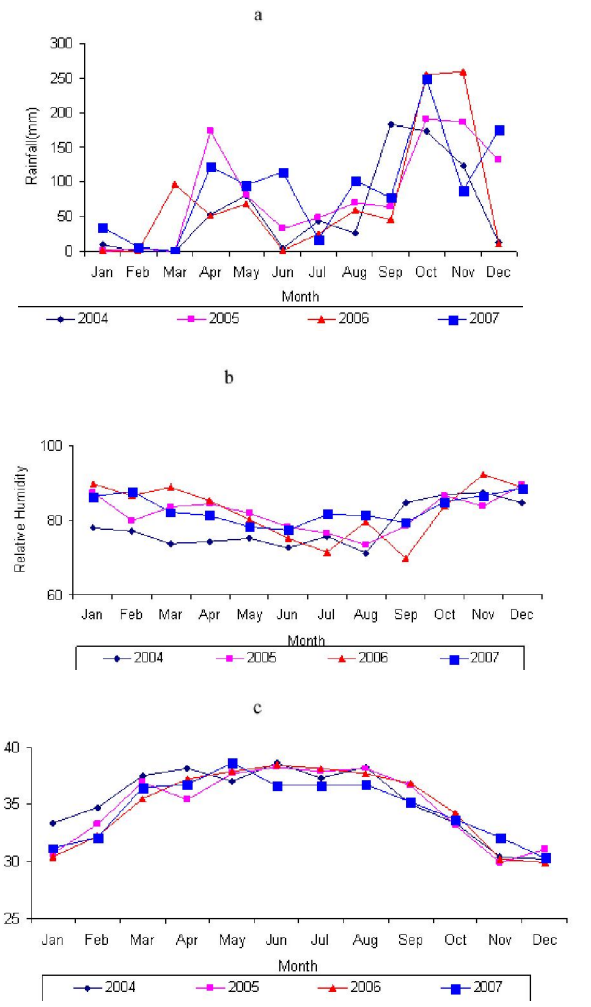

Figure 1. Rainfall (in mm) (a), relative humidity(in %) (b) and temperature (in ºC) (c) during study period from 2004 to 2007)

Moisture content of control plants of *A. vasica* expressed 64.047 ± 0.678% .Spider infested (AVW) leaves did not

Table 1. Rainfall (in mm), relative humidity (in %) and temperature (ºC) at different seasons of study period.

Pest	Season	Rain fall	Relative Humidity	Temperature
AVC	February – April	0 – 52.8	74.5 – 77.2	34.7 – 38.2
AVG	July - September	48.2 - 183.2	81.8 - 84.7	38.2 – 36.8
AVM	November - January	34.8 - 259.6	89.9 - 92.4	32.1 – 33.4
AVS	October - November	255 - 259.6	87.1 - 92.4	32.1 – 34.2
AVW	May - June	5.2 – 81.4	72.6 – 75.3	37.0 – 38.7

AVC – *A. vasica* control , AVG – *A. vasica* infested by grasshopper , AVM – *A. vasica* infested by codling moth larvae, AVS – *A. vasica* infested by scale Insect , AVW – *A. vasica* infected by weavers Spider .

Table 2 . Qualitative phytochemical examination of various extracts of *A. vasica*

Plant constituent test / reagent used	EXTRACTS					
	P.E	B.E	C.E	A.E	E.E	W.E
Alkaloids						
a) Mayer's reagent	-	-	+	-	+	+
b) Dragendroff's reagent	-	-	+	-	+	+
c) Hager's reagent	-	-	+	-	+	+
d) Wagner's reagent	-	-	+	-	+	+
Carbohydrates & Glycosides						
a) Molich's Reagent	-	-	-	-	+	+
b) Fehling Solution	-	-	-	-	+	+
c) Barfoed's Test	-	-	-	-	+	+
d) Benedict's Reagent	-	-	-	-	+	+
e) Libermann-Burchard's Test	-	-	-	-	+	+
f) Legal's Test	-	-	-	-	+	+
g) Borntrager's Test	-	-	-	-	+	+
Phytosterols						
a) Libermann's Sterol Test	+	-	-	+	+	-
b) Libermann – Burchard Test	+	-	-	+	+	-
Fixed oil & Fats						
a) Spot Test	+	+	-	-	-	-
b) Saponification Test	+	+	-	-	-	-
Saponins						
a) Foam Test	-	-	-	-	+	+
b) Haemolysis Test	-	-	-	-	+	+
Phenolic compounds & Tannis						
a) with Ferric Chloride Solution	-	-	-	-	-	+
b) with Gelatin Solution	-	-	-	-	-	+
c) with Lead acetate Solution	-	-	-	+	+	-
d) with Aqueous bromine Solution	-	-	-	-	+	+
Proteins & Amino acids						
a) Millon's Reagent	-	-	-	-	+	+
b) Biuret Test	-	-	-	-	+	+
c) with Ninhydrin Reagent	-	-	-	-	+	+
Gums & Mucilages						
a) Alcoholic Precipitation test	-	-	-	-	-	+
b) Molisch's Test	-	-	-	-	-	+
Flavones & Flavonoids						
a) with Aq.NaOH	-	-	+	-	+	+
b) with Con.H_2SO_4	-	-	+	-	+	+
c) with Mg.+ HCl	-	-	+	-	+	+

+ : Positive, - : Negative, PE - Petroleum ether Extract, BE – Benzene Extract, CE – Chloroform Extract, AE – Acetone Extract, EE – Ethanolic Extract, WE – Water Extract

Table 3. Ash value (in %) of healthy and pest infested leaves of *A. vasica* collected from Virudhunagar and Theni

Name of Sample	Total Ash	Acid Insoluble Ash	Water Soluble Ash	Sulphated Ash
Virudhunagar				
AVC	12 ± 0.048	1.058 ± 0.023	0.78 ± 0.037	10.22 ± 0.037
AVG	80.52 ± 0.037	2.0 ± 0.070	4.18 ± 0.096	73.96 ± 0.143
AVM	77.82 ± 0.176	4.048 ± 0.005	6.454 ± 0.009	66.44 ± 0.009
AVS	74.62 ± 0.297	3.0 ± 0.170	5.06 ± 0.180	66.92 ± 0.288
AVW	80.26 ± 0.242	3.0 ± 0.170	5.26 ± 0.258	69.62 ± 0.297
Theni				
AVC	9.76 ± 0.224	0.82 ± 0.037	0.78 ± 0.037	9.02 ± 0.156
AVG	74.04 ± 0.290	1.28 ± 0.111	3.16 ± 0.188	69.94 ± 0.378
AVM	72.04 ± 0.186	2.936 ± 0.118	5.154 ± 0.145	64.00 ± 0.315
AVS	76.8 ± 0.254	2.92 ± 0.124	4.52 ± 0.159	69.6 ± 0.367
AVW	75.0 ± 0.262	1.96 ± 0.186	4.22 ± 0.185	70.6 ± 0.509

Table 4. Extractive value of healthy and pest infested leaves of *A. vasica* collected from Virudhunagar and Theni

Name of Sample	Petroleum Ether extract	Benzene extract	Chloroform Extract	Acetone Extract	Methanol Extract	Ethanol Extract	Water Extract
Virudhunagar							
AVC	17.2 ± 0.07	1.46 ± 0.05	1.52 ± 0.037	6.68 ± 0.037	2.18 ± 0.19	2.72 ± 0.04	4.02 ± 0.02
AVG	1.46 ± 0.024	1.82 ± 0.037	3.08 ± 0.037	8.08 ± 0.037	5.6 ± 0.07	7.54 ± 0.05	17.76 ± 0.05
AVM	1.44 ± 0.05	4.16 ± 0.05	5.46 ± 0.05	7.66 ± 0.04	10.66 ± 0.05	7.56 ± 0.05	18.58 ± 0.06
AVS	1.1 ± 0.044	2.48 ± 0.037	4.38 ± 0.037	5.72 ± 0.037	6.48 ± 0.04	7.1 ± 0.04	16.24 ± 0.05
AVW	1.76 ± 0.05	1.72 ± 0.037	3.8 ± 0.031	7.42 ± 0.037	6.06 ± 0.05	7.48 ± 0.06	15.48 ± 0.05
Theni							
AVC	19.1 ± 0.044	1.76 ± 0.024	1.74 ± 0.05	7.78 ± 0.073	2.58 ± 0.037	3.12 ± 0.037	5.46 ± 0.024
AVG	1.52 ± 0.073	2.86 ± 0.024	4.08 ± 0.037	9.02 ± 0.037	6.08 ± 0.037	8.06 ± 0.04	18.7 ± 0.054
AVM	1.52 ± 0.073	5.22 ± 0.058	6.56 ± 0.04	8.62 ± 0.037	5.98 ± 0.037	8.76 ± 0.024	18.64 ± 0.06
AVS	1.76 ± 0.024	3.18 ± 0.037	5.72 ± 0.037	6.5 ± 0.037	6.78 ± 0.058	7.84 ± 0.024	17.5 ± 0.054
AVW	1.78 ± 0.037	2.66 ± 0.05	4.84 ± 0.024	8.43 ± 0.037	6.42 ± 0.037	8.1 ± 0.044	16.5 ± 0.031

AVC – *A. vasica* control , AVG – *A. vasica* infested by grasshopper , AVM – *A. vasica* infested by codling moth larvae, AVS – *A. vasica* infested by scale Insect, AVW – *A. vasica* infected by weavers spider.

Extractive values in control and AVW sample showed a wide range with petroleum ether extract. Aqueous extract of AVC gave a value equivalent to acetone extract. In the infested plants, extractive value was higher which ranging from 17.76 to 18.58 % .

Table 5. Estimation of chlorophyll in healthy and pest infested leaves of *A. vasica*

Name of Sample	Chlorophyll a (mg/gm)	Chlorophyll b (mg/gm)	Carotenoid (mg/gm)	Total Chlorophyll (mg/gm)
AVC	1.616 ± 0.0150	1.582 ± 0.0152	0.1146 ± 0.0001	3.24 ± 0.116
AVG	2.555 ± 0.0273	1.855 ± 0.007	0.121 ± 0.006	4.41 ± 0.008
AVM				
I Instar	2.447 ± 0.007	1.171 ± 0.036	1.092 ± 0.005	3.61 ± 0.013
II Instar	2.897 ± 0.004	1.463 ± 0.006	1.141 ± 0.006	4.36 ± 0.010
III Instar	2.630 ± 0.006	1.349 ± 0.004	1.042 ± 0.001	3.97 ± 0.005
IV Instar	3.030 ± 0.001	1.644 ± 0.023	1.252 ± 0.002	4.69 ± 0.011
AVS	2.552 ± 0.015	1.855 ± 0.003	0.1109 ± 0.0002	4.41 ± 0.013
AVW	1.674 ± 0.006	1.351 ± 0.005	0.172 ± 0.002	3.03 ± 0.006

AVC – *A. vasica* control, AVG – *A. vasica* infested by grasshopper, AVM – *A. vasica* infested by codling moth larvae, AVS – *A. vasica* infested by scale Insect, AVW – *A. vasica* infected by weavers spider .

Table 6. Estimation of phytochemicals (mg/g) in healthy and pest infested leaves of *Adhatoda vasica*

Phytochemicals	AVC	AVG	AVM	AVS	AVW
Glucose	1.92 ± 0.011	1.154 ± 0.038	0.978 ± 0.003	3.012 ± 0.022	1.34 ± 0.005
Amino acid	3.26 ± 0.153	10.76 ± 0.074	13.82 ± 0.066	31.2 ± 0.583	4.12 ± 0.037
Protein	42.14 ± 0.035	11.42 ± 0.010	14.28 ± 0.008	24.28 ± 0.008	33.57 ± 0.019
Phenol	0.0167 ± 0.0004	0.0244 ± 0.0006	0.0153 ± 0.0007	0.0254 ± 0.0001	0.0292 ± 0.0005
Tannin	0.310 ± 0.007	0.374 ± 0.007	0.114 ± 0.006	1.346 ± 0.007	0.230 ± 0.010
Flavonoid	0.380 ± 0.004	0.382 ± 0.002	0.387 ± 0.001	0.379 ± 0.039	0.378 ± 0.004
Anthocyanin	0.062 ± 0.002	0.256 ± 0.002	0.088 ± 0.003	0.173 ± 0.003	0.091 ± 0.003

show alter in the content, whereas insect infested AVG, AVM, AVS samples had 79.152 ± 2.227 %, 76.15 ± 1.578 % and 72.389±2.334 %. *Adhatoda vasica* plant had a high content of phytoecdusteroids, fat which could be extracted in petroleum ether. Alkaloids and carbohydrates could be extracted in ethanol and aqueous extracts. Phenolic compounds and gums and mucilage were found in aqueous exracts. In healthy plant raised in irudhunagar. Phytosteroids, fixed oil and fat were absent but flavonoids, gums and mucilage were present in the ethanolic extract.

Total ash value of TDL showed higher percentage which prompted good curative value . Sulphated ash of healthy samples gave a low 10 % whereas all the pest infested leaves exhibited a higher percentage ranging from 66.44 % to 73.96 % . The total ash value of AVC was 12 ± 0.048 but the AVG sample showed 80.52±0.037. The sulphated ash content was maximum in all pest infested leaves. The ash value had a remarkable difference in AVC sample. The acid insoluble ash and water soluble ash were comparatively higher in pest infested sample than the healthy leaves. The extractive value of petroleum ether showed 19.1 ± 0.044 in healthy leaves, but the pest infested samples showed low percentage. The extractive value was higher in the aqueous extract of pest infested samples than the healthy leaves .Among the alcoholic extracts the extractive value of acetone shows a remarkable difference in AVG sample 9.02±0.037.

Significant differences of chlorophyll pigments in healthy and pest infested leaves were noticed . The total chlorophyll content was high in (4.69 ± 0.011 mg / gm) AVM, AVS , AVG had same value of 4.41 ± 0.008 mg / gm and in the rest of other samples the total chlorophyll were in the range of 4.36 ± 0.010 to 3.03 ± 0.006 . However chlorophyll 'a' was significantly higher in all tested materials than chlorophyll 'b' . The carotenoid was high in AVM (1.141 ± 0.006 mg / gm) I instar .The lowest content was noticed in AVM of III Instar (0.092 ± 0.005 mg / gm).

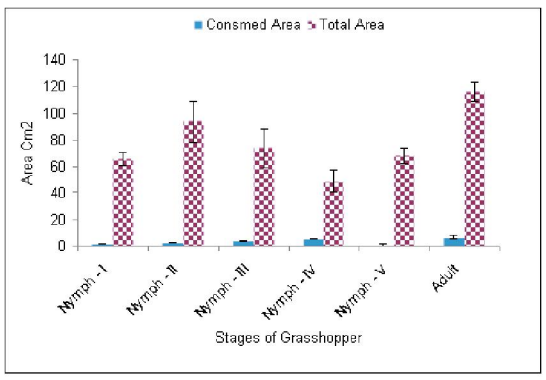

Figure 2. Damaging potential of *M.sanguinipes* on *A. vasica* leaves

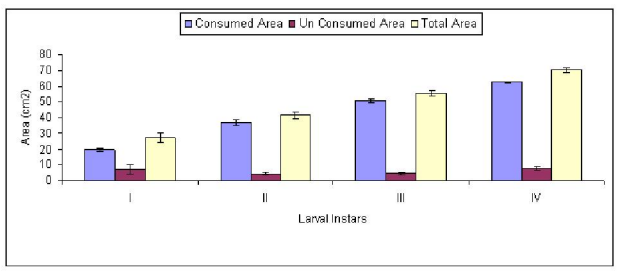

Figure 3. Damaging potential of *C.pomonella* on *A. vasica* leaves

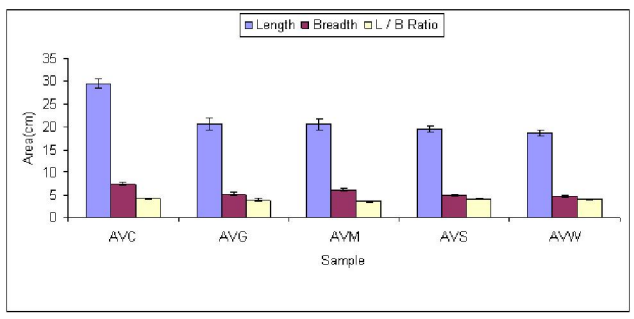

Figure 4. Length , breadth of healthy and pest infested leaves of *A. vasica* .

The highest sugar content 3.012 ± 0.022 mg/gm was recorded in AVS . The lowest sugar content 0.978 ± 0.003 mg/gm was recorded in AVM. There was tremendous increase in the amount of total free amino acid in AVS was 31.2 ± 0.583 mg / gm where as the lowest amino acid content was recorded in AVC . 3.26 ± 0.153 mg/gm . AVC showed highest protein content 42.14 ± 0.035 mg/gm . AVG showed lowest protein content 11.42 ± 0.010 mg / gm. AVW had maximum phenol content 0.0292 ± 0.0005 . AVM had minimum phenol content 0.0153 ± 0.0007 mg / gm . The AVS possessed highest tannin conternt 1.346 ± 0.007 mg / gm .Lowest phenol content 0.114 ± 0.006 mg/gm was recorded in AVM . Flavonoid content was same in all tested samples. The anthocyanin content 0.256 ± 0.002 mg / gm was higher in AVG . The lowest content of anthocyanin found in AVC 0.062 ± 0.002 mg / gm.

DISCUSSION

Adhatoda vasica Nees a hedge herb which has been a trap crop for many useful productive fruit trees. As a valuable medicinal herb, the plant traps four types of common pests in four different seasons except three months starting from February to April showed the lowest rainfall an ideal relative humidity (74.5 % - 77.2 %) and high temperature (34.7^{o}C – 38.2^{o}C). This season had been taken over by the weaver spider pest time with a minimum rainfall, relative humidity and optimum temperature. Grasshopper had chosen the July – September season with a medium climate and nominal damage to the medicinal plant. After this October – November and November – January seasons, Saissetia scales and caterpillars of moth destroy the foliage of this antiinflammatory and analgesic plant.

Since October – November seasons have recorded heavy rainfall up to 259.6 mm in almost all the few consecutive years damaging potential was high. The peak feeding stages of grasshoppers nymph IV stages exhibiting very heavy consumption almost denuding the plant. The length of the leaves of infested leaves got reduced and a L/B value showed a decrease from the healthy leaf which was 4.14 ± 0.181 as against 3.941 of AVW infested leaves. In general crude fibre content was high in flowering season whereas in *A. vasica* irrespective of the flowering season, scale insect infestation altered the crude fibre content.

Flavonoids, a group of plant polyphenolic compounds which occur both in the free state and as glycosides. Most of are O - glycosides but a considerable number of flavonoids are C - glycosides and possess broad biological properties. Flavonoid compound was isolated from the ethanolic extract of leaves. Isolated flavonoid, having good antioxidant activity against synthetic free radicals like DPPH and Nitric oxide. Ash values showed a marked difference in healthy and infested leaves. The healthy leaves showed low content of total ash whereas infested leaves showed around 72% to 76.8% sulphated ash.

Extractive value was higher in all the pest infested leaves compared to the healthy plants. Since most of the phytochemical and pharmacological studies water extracts were used, however the aqueous extractive value decided the efficiency. Ethanol extracts would usually proove good but such results could be interpreted for human clinical trials. Hence extractive values have been great importance. The problem of pest control was perennial and it needed effective pest management rather than pesticide usage because there was always a danger of pesticide poisoning in addition to the commercial loss on quality and quantity production. The pH rose up to 7.5 when cough syrup was formulated with pest infested leaves of *A. vasica*.

Adhatoda vasica, a crude plant drug which was highly valuable in clearing phlegm and lung problems was found to be infested with seasonal pests. Infested leaves showed high ash value out of which sulphated ash content was almost 80 % and more. This possibly interfered with the curative value of the green drug leading to allergy. Extractive value of extracts Petroleum ether got reduced in infested leaves. In Acetone, Benzene, Chloroform, Ethanol, Methanol and Water extracts the extractive value increased showing extra salts and acids.

The study was significant since the curative value was greatly modified by pest infestation. Further results in animal models could go a long way in choosing the right type of drug in the suitable season without side effects. The study would add to the supplementary value in manufacturing natural drugs without side effects (Jhansi Lakshmi, *et al*, 2005).

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Biopesticides Networking Programme (BNWP)

C/O Crop Protection Research Centre (CPRC),
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Biopesticides are living organisms (natural enemies) or their products (phytochemicals, microbial products) or byproducts (semiochemicals) which can be used for the management of pests (microorganisms – bacteria, fungi, protozoa, virus and viroid; macroorganisms – invertebrates and vertebrates) that are injurious to plants. Biopesticides have an important and increasing role in crop protection, although most commonly in combination with other tools including chemical pesticides as part of Bio-intensive Integrated Pest Management (BIPM). World-wide use of biopesticides has been rapidly increasing.

The initiation of Biopesticides Networking Programme (BNWP) has been suggested during the panel discussion of the Second Biopesticides International Conference (BIOCICON 2009) held at St. Xavier's College (Autonomous), Palayamkottai 627 002, Tamil Nadu, India during November 26 to 28, 2009.

The mission of the BNWP is to encourage and promote the Ecofriendly Pest Management through production, formulation and practicing of Biopesticides all over the world. To achieve this mission, the BNWP is pursuing the following major goals:

1. To bring the data-base of students, researcher scholars and scientists involved in the biopesticide research, small and large scale production of biopesticides, formulation, and application under laboratory, controlled cage/glass house and field level.
2. To establish forums on various aspects of biopesticides to discuss research, commercialization and utilization.
3. Enhancement of technical skills and capacity build-up of students, researchers and scientists through inter- and intra-exchange programme in voluntary manner.
4. Establishment and exploration of national and international collaborations in biopesticide research.
5. Publication of magazines, bulletins, e-bulletins, e-newsletters and books – in order to promote new technologies related to biopesticides in a form available and easily accessible to the farmers, students, researchers and scientists.
6. To encourage biopesticides research and development, young researcher award, senior researcher award, life time award, extension worker award, best biopesticides manufacturer award are constituted.
7. Conducting awareness programs among the growers and consumers by seminars, workshops, audiovisual media and literature regarding the hazardous effects of chemical fertilizers and pesticides; and possibilities of introducing principles of sustainable agriculture as an alternative.

Interested Scientist / Researchers / Students / Industrial Peoble / Farmer / Public can contact Dr. K. Sahayaraj, Director, Crop Protection Research Centre (CPRC), St. Xavier's College (Autonomous), Palayamkottai – 627 002, Tamil Nadu, India, Phone: 0462-4264376, Fax: 0462 2561765, Cell: 9443497192, E-mail: ksraj42@gmail.com for more details or log on in the website, (www.bionep.com)