BIO EFFICACY OF PURIFIED ZINGIBERACEAE LECTINS AGAINST TEA PEST AND PATHOGEN

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Abstract : Tea is being exposure to number pests and pathogens. Broad range of chemicals is also being used to overcome their attacks. Recent awareness of pesticide residues in soil flora as well quality of tea leaves turns focus on search of biological methods with safer to human health. Based on reviews lectins, especially mannose specific lectins of monocots were found to be safer and a promising source to treat various insect orders. In the present study for the first time an attempt was made with zingiberaceae lectins against important tea pest red spider mite (*Oligonychus coffeae* N.) and fungal pathogen *(Exobasidium vexans* M.) causing the disease blister blight through *in-vitro* bio assay. Ovi-positional deterrence was also calculated. These results revealed the efficacy of lectins at different levels.

Index Terms **- Tea, lectins, red spider mite, blister blight, zingiberaceae.**

1. INTRODUCTION

Tea plants are cultivated in tropical and subtropical climates and regions in which precipitation is coordinated according to months, summers and winters are lukewarm in addition to sour and humid land structure. Soil pH of 6.0 is preferable for tea cultivation when pH between the ranges 4.5 - 5.5 favors growth of this plant [1]. Tea is known as popular and oldest beverage with strong anti-oxidative agents, hence it is considered as nature's gift. The presence of a number of biochemical constituents as well therapeutic important constituents especially catechins [2] are making them as effective against toxic chemicals, free radicals and carcinogens [3]. The perennial habit of this plant, peculiar cultural conditions and humid climate of the tea growing areas are highly favorable for disease development.

Being a monoculture crop tea provides a stable microclimate for a number of pests and disease. Tea is grown in about 42.7 million ha (Tea Board India annual report 2015-16) the plants are attacked by a variety of herbivores and the profile of pests vary from region to region. Even though the number of pests recorded worldwide about 300 species of insects and mites as well 58 pathogenic fungi is recorded from Indian tea plantations. Mites are becoming serious pests of tea and they damage the green tissues of leaves, thereby reducing the photosynthetic efficiency resulting in yield reduction. Pink mite (*Acaphylla theae*), purple mite (*Calacarus carinatus*), yellow mite (*Polyphagotarsonemus latus*), palemite (*Acaphyllisa parindiae*) and scarlet mite (*Brevipalpus australis*) and red spider mite (*Oligonychus coffeae*) are considered as major pests and each is having a different mode of infestations (www.upasitearesearch.org). Red Spider Mite (RSM) causing serious crop damage during the past two decades and it comes under the order Acarina. In India this particular pest was initially recorded in coffee by [4], later in 1868, it was observed in tea. Infestation by RSM starts along midrib and veins initially and gradually spreads to the entire upper surface of leaves (Fig.1). Nymphs and adults of RSM lacerate cells are producing minute characteristic reddish-brown marks on the upper surface of mature leaves, which turn red in severe cases, resulting in crop losses up to 18% [5].

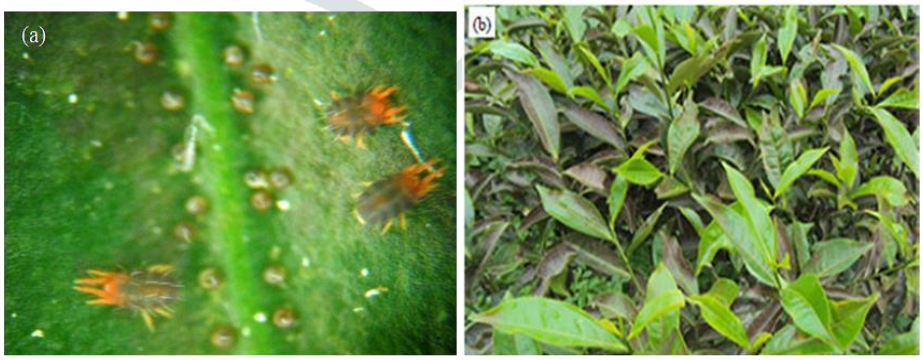

Figure. 1 : (a) individual red spider mites with their eggs and (b) mite infested leaves

Leaf temperature and light penetration within tea bushes also influence mite distribution; *O. coffeae* prefers the middle zone of the bush because of optimum temperatures associated with plant shading [6]. High temperatures, dry conditions and the absence of shade are favorable to the outbreak of this pest. Developmental stages include six-legged larva, protonymph, and deutonymph. Each developmental stage is followed by a quiescent stage and life cycle completed in 10-14 days. Life history and control measures of red spider mite had been reported earlier by several authors [7, 5, 8]. Moreover, tea also has been exposed to the number of pathogens. Majority of the tea diseases are of fungal origin and few are the bacteria and one each of virus and alga. There are 400 pathogens were described, irrespective of its casual agents and parts affected, the disease symptoms obvious as debilitation, defoliation and sometimes death of the bushes [9]. Blister and grey blight are the pathogenic leaf diseases which affect the crop shoots that have been weakened by improper care or adverse environmental conditions.

Blister blight disease caused by the fungal pathogen, *Exobasidium vexans* M. [10], an obligate parasite belonging to the class Basidiomycetes, of family Exobasidiaceae affects the shoots of tea. Cloudy, wet weather favors their infection. Small, pale- green or pale-yellow and translucent oily spots are the preliminary indication of this disease. On the upper side of the leaf, the spots slowly become sunken into a shallow depression; at the same time on the venal under-side, they become correspondingly convex, forming the typical blister lesion (Fig.2). The upper concave surface of the lesion is smooth and shiny, whereas the lower convex surface is at first dull, then grey and finally pure white, due to a dense seems like velvety growth on which the spores are produced. Spore will take 9-15 days of incubation once it affects the new leaf. The pathogen completes its life cycle in a short span of 25 days when the weather conditions are conducive. The disease attains maximum outburst during monsoon months, i.e. during June-December then start to vanish during summer seasons [11].

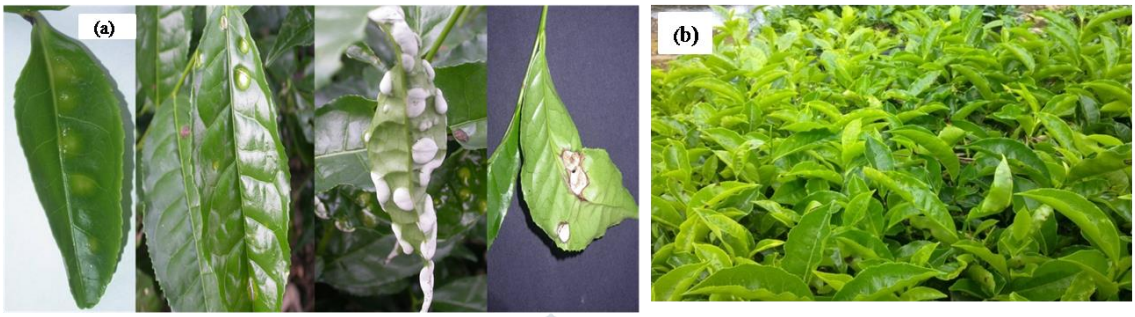

Figure. 2 : (a) different stages of blister blight fungal infection and (b) blister infested leaves

Continuous infestation of pest and pathogens on the tender and succulent leaves of this beverage crop are major threads for their commercial production. In general use of traditional and organophosphate pesticides against insect pest management has resulted in the decline other beneficial insects, secondary pest outbreak and emergence of insect strains resistant to pesticides. Likewise, these strategies had driven to the conservation of biological controls (Natural enemies, predators) as well leads to awareness over the finding of non-toxic and environmentally friendly alternatives to chemicals. Bio-pesticides are being used as an alternate strategy to chemical pesticides as they are target specific and environmentally friendly due to their higher selectivity and biodegradable nature. Botanical insecticides were tested as biocontrol agents since these are the insect toxins derived from the plant species. In tea number of botanical products were tested for their pesticidal/insecticidal activities [12- 14]. However continuous application of all the control measures may lead pest/pathogen to develop resistance which turns to search for better-controlling agents. Protease inhibitors, amylase inhibitor and lectins were considered as new insect control agents due to their effectiveness against pests and they were being evaluated for their ability to confer the broad-spectrum insect resistance through transgenic crops [15].

In recent years lectins were gaining more attention due to their wide range of resistance towards pests, fungal, bacterial and viral pathogens besides nematodes [16]. Lectins represent a various group of oligomeric proteins varying in size, structure, molecular organization and their carbohydrate-binding sites. Structural analysis of lectins showed slight variations in primary structure and carbohydrate-binding specificity but differences in their biological activities [17]. The ability of lectins to bind glycoconjugates from other organisms provides a strong suggestion that lectins are involved in the plant's defense strategy. The monocot mannose-binding lectins (MMBLs) have received much interest and become an important tool in plant protection and plant biotechnology since their genes confer resistance against sucking insects and nematodes [18]. Till date, seven monocot plant families were well documented for their insecticidal activity and their structure analysis revealed that they are structurally and functionally related ones and potential mannose – recognizers [19]. Zingiberaceae is one among the monocots constitutes medicinally a vital group of rhizomatous medicinal and aromatic plants characterized by the presence of volatile oils and oleoresins which is not explored. In addition to that this family includes economically important genera including *Curcuma, Zingiber, Alpinia* and *Elettaria* are reported as potential medicinal plants. A number of investigations have been done on medicinal aspects of zingiberous plant species by means leaf extracts and their important volatile compounds. However, sporadic information on isolation and characterization of proteins and their molecular characterization aspects paves the way to explore them in this concern.

With this above given background and considering the defense properties of the plant lectins especially mannose-specific lectins, the present study was taken as a chance to isolate new lectins from the easily available edible plants. These findings will help to consider these lectins as a novel member of the monocot mannose-binding lectins. Evaluation of their insecticidal/fungicidal activity was done against as above which are the major threats to tea productivity. This study may form a base line for the evaluation of lectins insecticide and fungicide property and also in developing of resistant variety with the safest source of the transgene in crop disease management.

2. MATERIALS AND METHODS

We used Affinity chromatography in this study for the purification of lectins from tuber, rhizome and seed of *Alpina galanga*, *Curcuma longa*, *Zingiber officinale* and *Eletteria cardamom* respectively. This technique exploits the bio specific relationship between a protein and a ligand, to specifically select out a desired protein from a crude mixture, essentially in a single step. Single band purified lectins were subjected to agglutination assay, which is the preliminary characterization of lectins. Purified lectins were subjected to PAGE analysis for calculate their subunit molecular weight as well physio-chemical characterization was done were detailed explained in previous publication [20].

2.1. Determination on bio-efficacy of lectins on RSM

2.1.1. Mass Multiplication of RSM

The RSM was maintained by the entomology division for the in vitro bioassay with lectins. Mass multiplication of RSM was done by collecting RSM from the tea fields of UPASI Experimental Farm at an altitude of 1050 m.am.s.l, Valparai, Tamil Nadu,

India and they were transferred onto one year potted tea plants (cultivar) grown under greenhouse conditions where the temperature was maintained at 25 ± 2˚C, 75 ± 5% RH and 16L: 8D photoperiod. Withered and dry leaves were regularly replaced.

2.1.2. *In-vitro* laboratory bioassay

Matured tea leaves (UPASI 9 clone) without any RSM population were collected from the field. Leaf discs of 2 cm diameter were placed on moist cotton in a Petri dish (9 cm diameter). Different concentration of (25, 50, 75 and 100 µg/ml of lectin solutions) was overlaid on the disc and allowed to dry at room temperature (Fig.3). Leaf discs overlaid with distilled water served as untreated controls. Ten adult RSM (less than 48 h old) were transferred from the stock onto lectin treated leaf discs. Each treatment was replicated in five and the Plates were maintained at the room temperature and results were observed after 24, 48, 72 and 96 hours under the binocular microscope (Olympus No.1220). The survival of individual mites was determined by touching each mite with a single hair brush and mites that were unable to walk at least the distance equivalent to their body length were considered as dead.

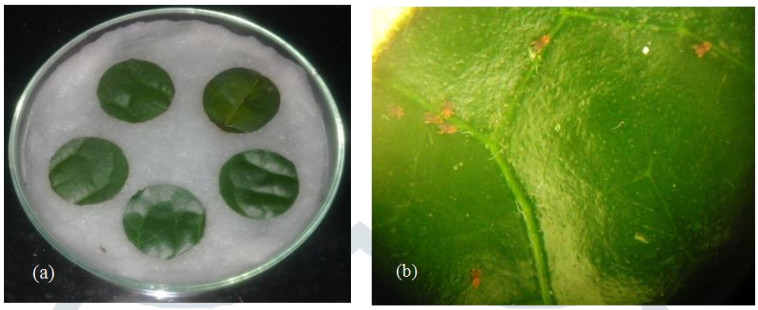

Figure. 3. (a)-RSM- bioassay setup, (b). adult mites on leaf disc

2.1.3. Ovi-positional Deterrence

Ovi-positional deterrence was studied by allowing the adult females (10 individuals /treatment) to lay eggs both on the treated and untreated control leaf discs. The degree of deterrence was assessed in terms of differences in the number of eggs laid by the females in control and treated leaves. Each treatment was replicated in five.

2.1.3. Statistical Analysis

Data generated were subjected to statistical analysis (ANOVA) and results presented in accordance with DMRT (SPSS, 10). The percent mortality was calculated using the "Abbot's correction analysis" (Abbott, 1925). The Discrimination Quotient (DQ) was calculated using the formula, DQ = C-T/ C+T where C, number of eggs on control leaves; T, the number of eggs on treated leaves.

2.2. Evaluation of lectin activity against germination of *Exobasidium vexans* M.

2.2.1. Harvesting of basidio spores

The blister blight pathogen spores were collected from the infected tea leaf using Bell-Jar method [21]. Basidio spores were collected from naturally infected blister lesions. For this purpose, fresh tea shoots with actively sporulating lesions of stage 7 were collected from the field in the evenings and the cut ends of the shoots were kept in sterile glass vials containing 1% glucose solution. A clean, sterile beaker was taken and kept below the leaf with a single active sporulating lesion. The mouth of the beaker was closed with the leaf in such a way that the lesion is in the beaker facing towards its bottom. A small glass plate was kept over the leaf with a small weight on it to keep the leaf intact. The setup was incubated overnight inside a bell jar under 100 percent relative humidity (Fig.4a). The spores released were deposited in the bottom of the beaker as a while patch (Fig.4b&c). The beaker was removed on the following day. The spore mass into a suspension with sterile distilled water and the spore concentration was adjusted to $1x10^6$ spores mL-1

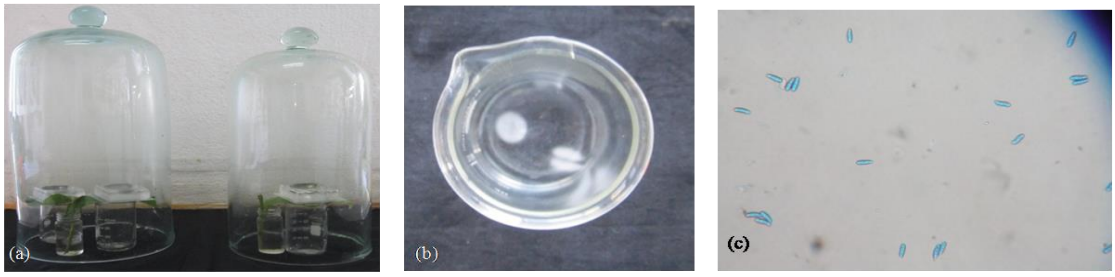

Figure. 4. Method of Basidio spore collection from blister lesion

2.2.2. Spore Germination Inhibition Assay

A 20 µl aliquot of spore suspension was added with 20 µl of different concentrations of lectin. Spores in distilled water and spores incubated with 0.1% glucose solution served as controls. The controls as well as treated spore suspensions were placed on separate slides in a moisture chamber for 24 hours of incubation. Then a drop of lactophenol cotton blue was placed on the spore suspension on the slides. The slides were examined under the microscope (40X) for recording the percentage of spore germination. The observations were recorded after 24, 48, 72 and 96 hrs. Five different microscopic fields were examined and the spore germination was calculated using the following formula,

Germination %= (Number of spores germinate)/ (Total number of spores) X 100

3. RESULTS AND DISCUSSION

The present study reports the purification and characterization of a lectin from rhizomes and seeds of Zingiberous plants, further their efficacy against tea major pest and pathogen was evaluated. Detailed purification procedure and their physio- chemical characterization were carried out before evaluation and it has published by us [20]. Purified lectin solutions were prepared at a different concentration of 25, 50, 75 and 100µg /ml then applied on tea leaf disc and allowed to dry at room temperature. Adult and healthy mites were selected from the mass multiplied trays by touching them. Five replicates were taken in each Petri plates then mites were allowed to feed the leaf disc and their activity was observed at time intervals. Leaf disc was placed upon the Petri disc supporting by the cotton. Cotton was watered frequently so that mites could not move lower side of the leaf disc and also help to feed treated leaf completely. Dead mites were counted to find the mortality caused at a different concentration, the mites which are not able to move at least their body length were considered as dead. In this treatment, all four lectins showed different mortality rates and they were shown in the following tables. Statistical expressions shown in all above tables denote mean of five replicates followed by ± standard error. A mean value followed by the same alphabet in a column do not differ significantly at P = 0.05 according to Duncan Multiple Range Test (DMRT).

Bio efficacy of the *Alpinia galanga* tuber lectin was shown in table 1. Among the four conc. tested, maximum mortality of 98% was achieved in the highest conc. of 100 µg/ml. about 80% mortality was observed at 24hrs with this higher concentration. As the concentration of lectin increased, mortality rate of RSM enhanced parallel. Incubation period also played an important role in RSM mortality. Hence, dose and time dependant mortality was clearly observed at 96 hrs. On the same experiment with *Curcuma amada* (mango ginger) rhizome lectin is presented in table 2. Mango ginger lectin showed 60% mortality after 4 days at the conc. of 100 µg/ml shows, it moderate activity against the tea pest. Lowest concentration tested (25 µg/ml) caused 34% mortality in 96 hrs.

Table. 1. Bio efficacy of *Alpinia galanga* tuber lectin (AGTL) on RSM adults

Dose (µg/ml)	% of mortality after (hrs)			
	24	48	72	96
Control	00 ± 0.0a	00 ± 0.0a	00 ± 0.0a	00 ± 0.0a
25	16 ± 2.4b	32 ± 3.7c	40 ± 3.1b	46 ± 2.4b
50	22 ± 3.7b	34 ± 2.4c	46 ± 2.4b	62 ± 2c
75	64 ± 2.4c	68 ± 3.7c	78 ± 3.7c	84 ± 2.4d
100	80 ± 3.1d	82 ± 3.7d	90 ± 3.1d	98 ± 2e

Note: Mean values followed by the same alphabet in a column do not differ significantly at P = 0.05 according to DMRT.

Table. 2. Bio efficacy of *Curcuma amada* rhizome lectin (CARL) on RSM adults

Dose (µg/ml)	% of mortality after (hrs)			
	24	48	72	96
Control	00 ± 0.0a	00± 0.0a	00± 0.0a	00± 0.0a
25	10 ± 0b	18 ± 2c	20 ± 0b	34 ± 2.4c
50	18 ± 2b	28 ± 2c	34 ± 2.4b	42 ± 2d
75	26 ± 2.4d	32 ± 2c	38 ± 2c	44 ± 2.4d
100	36 ± 2.4e	40 ± 3.1d	46 ± 2.4d	60 ± 3.1e

Note: Mean values followed by the same alphabet in a column do not differ significantly at P = 0.05 according to DMRT.

Cardamom seed lectin too has given a significant level of mortality at the concentration of 100 µg/ml. About 100 µg/ml of seed lectin caused 86% mortality after 96 hrs of observation as shown in the table 3. Like all above lectins, ginger rhizome lectins showed a time and dose dependant response. Hundred / cent percent mortality was observed by ginger lectin in the present study with 75and 100 µg/ml at 48 and 24 hrs of treatment respectively (table 4). However, 72% mortality resulted with in 24 hrs. The increase in mortality even up to 96 hrs implies that the above-tested lectins are stable and active at room temperature during the course of study.

The result obtained from the leaf disc bioassay indicated that among the different lectins tested *Alpinia* and ginger lectins were more effective against RSM adults than the other two lectins. LC50 of ginger and Alpinia is 22.3 and 23.8 respectively and caused 100% mortality at 100 µg/ml concentration after 4 days (table 5). All the above-mentioned plant lectins tested against RSM can be considered as acaricides against RSM. They caused significant mortality under laboratory conditions and have no phytotoxic effect on host plants. In addition to mortality, it has ovi positional deterrence also. The result of the ovi positional deterrence is presented in table.6. Based on the results ginger rhizome lectin showed more deterrence (0.99) followed by *Alpinia* tuber lectin (0.86). Lowest deterrence (0.19) was noticed in mango ginger rhizome lectin. Based on the results, test solutions were shown a different range of mortality effects on RSM. Prolong incubation helps to kill the pest increasingly.

Table.3. Bio efficacy of *Eletteria cardamom* seed lectin (ECSL) on RSM adults

Dose (µg/ml)	% of mortality after (hrs)			
	24	48	72	96
Control	00± 0.0a	00± 0.0a	00± 0.0a	00± 0.0a
25	10 ± 0b	22 ± 3.7b	34 ± 2.4b	36 ± 2.4b
50	16 ± 2.4c	26 ± 2.4b	36 ± 2.4b	42 ± 2b
75	36 ± 2.4c	44 ± 3.7c	56 ± 3.7c	62 ± 2.4d
100	42 ± 3.1d	56 ± 3.7d	72 ± 3.1d	86 ± 2e

Note: Mean values followed by the same alphabet in a column do not differ significantly at P = 0.05 according to DMRT.

Table.4. Bio efficacy of *Zingiber officinale* rhizome lectin (ZORL) on RSM adults

Dose (µg/ml)	% of mortality after (hrs)			
	24	48	72	96
Control	00 ± 0.0a	00 ± 0.0a	00 ± 0.0a	00 ± 0.0a
25	20 ± 3.1b	32 ± 3.7b	52 ± 3.7b	60 ± 3.1b
50	60 ± 3.1c	80± 3.1c	86 ± 2.4c	92 ± 3.7c
75	90 ± 3.4d	100 ± 0.0d	100 ± 0.0d	100 ± 0.0d
100	100 ± 0.0d	100 ± 0.0d	100 ± 0.0d	100 ± 0.0d

Note: Mean values followed by the same alphabet in a column do not differ significantly at P = 0.05 according to DMRT.

Table.5. LC50 value of plant lectins

Lectin source	Concentration tested (µg/ml)	LC50 value (µg/ml)
Alpinia galanga tuber	100	23.8
Curcuma amada rhizome	100	122.2
Eletteria cardamom seed	100	47
Zingiber officinale rhizome	100	22.3

Table.6. Effect of lectins on ovi positional deterrence of female RSM

Source, Maximum Concentrations tried (100µg/ml)	No. of eggs laid at 96 h (Mean ± SE)*	DQ value
AGTL	5±3.8	0.88
CARL	61.2±1.20	0.19
ECSL	51±0.22	0.76
ZORL	00±0.0	0.99
Control	126.4±3.47	0

Note: *Values represent mean of five replications ± SE. A total number of adult females exposed for egg laying (N = 10).

Insecticidal properties of the lectins have been known against a number of insect orders [22, 23]. Lectin which may be most effective against some group of insect pest will be less effective / not effective against another group of the pest. For instance, purified phyto hemagglutinin (PHA) is not toxic to cowpea weevil larvae when fed through diet at levels as high as 1%, but dose-dependent mortality was observed when fed to the potato leaf hopper (*Empoasca fabae*) [24]. The mortality shown by RSM which is the result of dose and time and the concentration of lectin increased mortality rate of RSM increased concurrently. In 2010, [25] reported that garlic aqueous extract; neem and pongam kernel aqueous extracts were effectively used for the control of RSM. [26] and their crew in 2008 reported the effectiveness of garlic bulb lectin on RSM proved its toxicity against the pest. Similar to garlic bulb lectins, other lectins which are present in plants like in *Zingiber officinale* and *Curcuma longa* also come under monocot mannose binding lectins, due to their specificity towards mannose. Crude extracts and essential oils of zingiberous plant species traditionally used as insecticides, but there was no report available on insecticidal property as protein level. Lectins isolated from our source plants are found to be mannose specific. The interaction of lectin against RSM depends on the number of carbohydrate binding sites present in the body parts of mites and their strength of interaction with lectin. The reason behind this mechanism may be due to the interaction of lectins with the carbohydrate moieties on the surface of the insect's body or binding of lectins to the glycosylated digestive enzymes. [27] explained that monocot mannose binding lectins having tetrameric structure showed more potent insecticidal property and antifungal activity than the dimeric or trimeric lectins due to their ability to interact strongly with complex glycol conjugates.

The binding affinity of mannose binding lectins differs from each other. The statement was supported by ASA and GNA binding studies conducted by [28]. Sugar specific studies of ASA and GNA using strong glycol protein invertase showed increased ASA binding. This was due to the presence of α-l-2-linked mannose residue at the non-reducing ends of high mannose oligosaccharides in invertase, while GNA recognizes only terminal α-1-3-linked mannose residues. This suggests that the mannose binding affinity of ASA on the insect gut will differ from that of GNA according to the structure of the mannose oligosaccharides present there. Probit analysis was carried out to statistically denote the actual LC50 value of the effective lectin. This will be useful for the toxin expression in a transgenic situation for protecting the tea crop from the RSM. The LC50 values of the all four lectins were predicted for adult females at 96 hrs post-treatment. It was 23.8, 122.2, 47 and 22.3 respectively. Ginger showed the lowest LC50 (22.3) against RSM under laboratory conditions in comparison to the other lectins while mango ginger lectin showed the highest LC50 (122.2).

Bioassay experiment with lectins from *Allium sativum* leaf, *Diffenbachia* leaf and *Colocasia* tuber on *Aphis craccivora* was carried out by [29] and their LC50 value found as 0.150, 0.184 and 0.212 nanomoles respectively. Among three lectins garlic leaf found to be more toxic. Whereas [26], reported the LC50 values 12.4+1.9 µg/ml for garlic bulb lectin against RSM which differs considerably might be due to the different protocols followed for purification as well as modified bioassay set up. According to our in-vitro bioassay results, ZORL was reported to be an effective toxic lectin to RSM. Mannose-specific lectins from different plant families have tested again the various range of insect orders *A.sativam* leaf agglutinin was reported to be toxic against homopteran insect pests *Lipaphis erysimi* and *Dysdercuscingu* latus through *in vitro* assay, by [30]. In 2006 [31] compared the effects of GNA, ASAL and the *A. cepa* agglutininon nymphs of the mustard aphid (*L.erysimi*) through artificial diets assay and reported that ACA is more toxic than GNA and ASAL. Comparative analyses indicated that GNA-related lectins found in species of Alliaceae are substantially more active than GNA, in terms of specific agglutination activity [32]. The additional carbohydrate binding domain present in garlic lectin which is not present in the GNA is responsible for the control of insects at a lower dose [30].

Mode of action of lectin on insects varies according to its structure. However the lectin effective against some insect may not be effective against a group of insects, since the glycoprotein structures are different among them. In the present study, we noticed

oozing out of body fluid from mite body that leads to death [33]. Significant shrinkage of the body of the mites was observed prior to its death and also the slow movement of RSM was noticed after 24 hrs in test plates when compared to the control irrespective of the lectin sources used (Fig.5). This clearly indicates the interference of lectin with the normal behaviour of RSM. The application of *P. fluorescens* culture on RSM showed reduced mobility and cessation followed by the oozing of the body fluid [34]. The *Allium sativam* agglutinin (ASA) treated nymphs of RSM survived over three days at 25 µg and 30 µg of ASA. When placed back on the control leaf pieces, they could not attain adulthood even after seven days of incubation while the nymphs on control leaves turned into adult mites within seven days [26].

Figure.5. RSM- lectin treatment observation at different time intervals (24 -96 hrs), a-control disc, b-oozing out of body fluid and c-body shrinkage observed on treated disc

Artificial diet bioassay conducted by [35] against red cotton bug using *A.sativam* agglutinin (ASAI, ASAII), *C. esculenta* agglutinin and *D.sequina* agglutinin showed the strongest toxicity of garlic lectins ASA I & II than other two lectins. Besides they affected the phenotype in the offspring with smaller and paler nymphs compared to control. Transgenic oilseed rape with the *Pisum sativum* lectin (mannose / glucose-specific) witnessed reduced growth in pollen beetle (*Meligethes aeneus*) larvae [36]. A laboratory bioassay using carmine spider mites (*Tetranychus cinnabarinus*) showed improved pest resistance in the transgenic papaya plants [37].

Since RSM has a short life span, the population density will reach its peak in shorter duration which will result in severe crop loss. Hence, control of their population needs considerable attention. Ovi-positional deterrence is considered to be a significant factor in RSM control. When the leaves were overlaid with test solutions, adult mites showed discrimination among the treated leaves with respect to the number of eggs. RSM laid less number of eggs on lectin treated leaves than the untreated control, which clearly proved the interference of lectin in its ovi-positional behaviour. Discrimination quotient value ranged from 0 to 1 which is an index for determination of the effect of chemicals on the insect's ovi-positional behavior (table.6.). DQ value of 0.99 was achieved at 100 µg/ml of ginger lectin followed by 0.88 at 100µg/ml of alpinia showed more potent deterrence followed by cardamom seed lectin (DQ-0.76) at 100 µg/ml concentration. [13] obtained the DQ value of 0.94 and 0.91 when the RSM was treated with the aqueous extract of *Morinda tinctoria* and *Pongamiaglabra* at 7.5% concentration respectively.

DQ values of 0.88 and 0.96 were obtained when garlic bulb aqueous extracts and neem kernel aqueous extracts were sprayed on RSM, respectively [26]. Seed and leaf extracts of *Datura stramonium* L. decreased ovi-position in two-spotted red spider mite, *T. urticae* (Koch) [38]. Application of acetone extract of garlic bulb on leaves of *Phaseolus vulgaris* reduced the fecundity of *T. urticae* [37]. GNA expressed in transgenic plants at a high level, caused an adverse effect on mites, including the production of a low number of eggs, delay in egg laying and hatching [39]. Essential oil of *Elettaria cardamomum* was researched on repellent and ovi-position inhibition of *T. urticae*. The LC50 values of fumigant toxicity of this oil on adults and eggs of the two spotted spider mite were 7.26 and 8.82 µL/L air, respectively. Also, the LT50 value of essential oil at 45 µL/L air was 23.86 h and LT50 value of essential oil at 60 µL/L air was 9.01 h. In addition, different concentrations of the essential oil of *E. cardamomum* significantly affected ovi-position deterrence and repellence of adults [40]. With this bioassay experiment, it is found that four lectins can kill tea pest actively. Although the lectins were delivered different mortality rates, ovi positional deterrence this investigation will provide the initiative to explore these lectins and also other zingiberous members in this line for controlling tea pest.

In order to check the antifungal activity of plant lectins on *Exobasidium vexans*, the spores collected from the infected active lesion were incubated at different concentrations of lectin solutions and incubated up to 96 hrs. Every 24 hrs of incubation, the spores were observed under light microscope (40X) and the germination percentage was documented. All the four plant lectins isolated in the present study were used and all showed the anti fungal effect by inhibiting the spore germination of *E. vexans* in the applied concentration range (25-100 µg/ml). Tested concentration for each lectin was selected based on the RSM bio assay results and almost similar concentrations were tried for spore germination inhibition assay. All the lectins tested were found to be acting in a dose-dependent manner. Complete inhibition was not recorded in tested concentrations of lectins; however, a significant reduction in spore germination was recorded for all the tested lectins compared to the untreated control and positive standard (0.1% glucose).

Bio efficacy of AGTL is documented in table.7 Among the 25, 75 and 100 µg/ml concentrations tried, germination was noticed at all concentrations in 24 hrs and a lower percentage of germination was observed in 100 µg/ml (8.25%) where as the control (0.1% glucose solution) attained ~74% germination. When incubation time increased, the spores started germinating and reached 50% of germination in 96 hrs at 25 µg/ml. Spores incubated with sterile water attained 58.25% germination whereas spores incubated in control had reached the stage of matrix formation (Fig.6), which was considered as 100% germination. As above *Alpinia* lectin showed a significant reduction in the spore germination compared to the control. Bio efficacy of mango ginger lectin on spore germination of *E. vexans* is expressed in table.8 All the concentrations tested showed a significant reduction in germination at 24, 48, 72 and 96 hrs. Lowest germination of 17.5 % was observed at 100 µg/ml in 96 hrs.

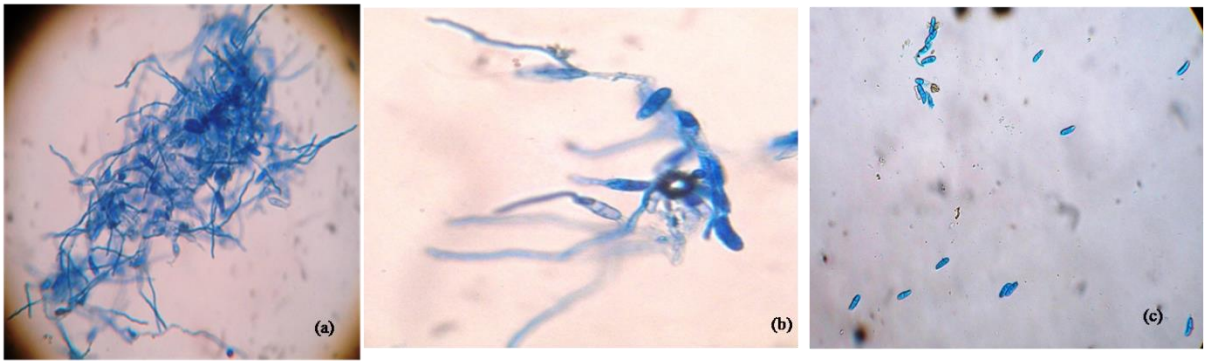

Figure.6. Observation of spore germination at different time intervals (24 -96 hrs) a – spores in 0.1% glucose , b- sterile water and c spores treated with lectin solution

Influence of cardamom seed lectin (CARL) on spore germination is given in table.9 about 10.25% of germination was observed at 100 µg/ml concentration at 96 hrs which is more or less similar to the germination of spores incubated with sterile water. A notable difference was observed between the concentrations as well as time periods (24 and 48 hrs). Until 48 hrs, germination was not increased in higher concentration. At 72 hrs, few spores were found to germinate and reach 15% in 96 hrs, and there was no much difference observed in concentrations 25 and 50 µg/ml. Effect of ginger rhizome lectin on spore germination is shown in table.10 10% of germination was observed at 100 µg/ml concentration in 24 hrs. Dose dependant response was observed for 24 and 48 hrs. But in 72 hrs and 96 hrs, there is no variation in the inhibiting effect between the concentrations. 14% of germination was seen in 100 µg/ml at 96 hrs. However all the four plant lectins tested in our present study showed the anti-fungal effect by inhibiting the spore germination of *E. vexans* under *invitro* conditions, the maximum percent of inhibition, 82% was noticed for AGTL and a significant level of inhibitions were seen for other lectins at various concentration range (25-100 µg/ml).

Table.7. Bio efficacy of *Alpinia galanga* tuber lectin on *E. vexans* spores

Dose (µg/ml)	% spore germination after (hrs)			
	24	48	72	96
0.1% glucose	26 ± 1.6c	42.5 ± 3.cd	84.25 ± 2.4d	100 ± 0d
Distilled water	12.12 ± 1.6b	19.33 ± 0.9b	30.4 ± 0.5c	58.25 ± 1.0c
25	35 ± 0.9c	39.75 ± 0.9d	43.5 ± 0.5cd	50 ± 0.8d
75	28.25 ± 0.4c	30.5 ± 0.8c	33.75 ± 2.2c	36.25 ± 2.04c
100	8.25 ± 0.4a	10.25 ± 0.6a	11.25 ± 0.76a	11.75 ± 0.4a

Table.8. Bio efficacy of *Curcuma amada rhizome* lectin on *E. vexans* spores

Dose (µg/ml)	% spore germination after (hrs)			
	24	48	72	96
0.1% glucose	26 ± 1.6d	42.5 ± 3.d	84.25 ± 2.4f	100 ± 0e
Distilled water	12.12 ± 1.6b	19.33 ± 0.9b	30.4 ± 0.5c	58.25 ± 1.0c
25	43± 1.26f	49.75 ± 0.6b	56.75 ± 0.7d	60.75 ±1.02d
75	35.25 ± 0.7e	40.5 ± 0.5b	41 ± 0.63c	42.25 ± 0.7c
100	6.75 ± 0.9a	7.5 ± 0.5a	11.25 ± 0.7a	17.5 ± 0.5a

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Table.9. Bio efficacy of *Eletteria cardamom* seed lectin on *E. vexans* spores

Dose (µg/ml)	% spore germination after (hrs)			
	24	48	72	96
0.1% glucose	26 ± 1.6b	42.5 ± 3d	84.25 ± 2.4e	100 ± 0e
Distilled water	12.12 ± 1.6b	19.33 ± 0.9b	30.4 ± 0.5c	58.25 ± 1.0d
25	27 ± 1.6b	34 ± 1.4c	39.5 ± 0.5c	42 ± 1.09d
75	29.75 ± 0.7b	37 ± 2.1c	41 ± 0.7d	43.75 ± 1.38d
100	10.25 ± 0.6a	11.5 ± 0.93a	14.5 ± 1.18a	15 ± 0.3a

Table.10. Bio efficacy of *Zingiber officinale rhizome* lectin on *E. vexans* spores

Dose (µg/ml)	% spore germination after (hrs)			
	24	48	72	96
0.1% glucose	26 ± 1.6b	42.5 ± 3c	84.25 ± 2.4d	100 ± 0d
Distilled water	12.12 ± 1.6a	19.33 ± 0.9a	30.4 ± 0.5b	58.25 ± 1.0c
25	17.75± 1.1a	27.5 ± 1.5c	33 ± 2.3b	53.25 ± 0.9c
75	13.25 ± 1.7a	22.5 ± 1.5b	31.25 ± 0.5b	34.5 ± 2.08b
100	6.5 ± 0.5a	11.5 ± 0.9a	14 ± 0.9a	14 ± 0.76a

Spore germination is one of the significant stages of fungal life cycle. Spores are dispersed in nature and thus inhibition of their germination plays an important role in reducing further development of the disease. Plant derived compounds are safer

alternatives to synthetic/chemical fungicides. Traditionally plant extracts have been used domestically for the control of pathogenic fungus. In 2007 [41] reported the total inhibitory effects of *Ocimum basilicum* L and *Allium sativum* L extracts on mycelia growth of *Colletotrichum gloeosporioides*. The extracts of *Vinca rosea*, Piper beetle, and *Azadirachta indica*, completely inhibited (100%) the spore germination of *Bipolaris sorokiniana* [42]. In this context, zingiberous plants are well known for controlling microbial pathogens. Fungi toxic constituent isolated from the essential oil of *Alpinia carinata* rhizome showed strong fumigant activity against *Rhizoctonia solani* [43]. Efficiency of nine essential oils of zingiberaceae species against three filamentous fungi was assayed by [44] and noticed considerable activity with a minimum concentration of 0.63µgµ-1. Anti microbial effect of Zingiber zerumbet rhizome proved against the bacteria *Vibrio parahemolyticus* with MIC value 128µg/ml [45]. *Alpinia galanga* and *Eleterria cardamomum* are also adding their points as antimicrobials [46].

There are only few plant lectins known to possess antifungal activity [47]. Inhibition of spore germination possibly occurs at a very early stage of the germination process, after the spores swell and before initiation of detectable germ tubes that lead to germination. In the present study, four mannose specific lectins were tested for their anti fungal activity against *E. vexans* spores by means of spore germination inhibition assay. The in vitro results revealed that the tested plant lectins effectively reduced the spore germination of *E. vexans* at various concentrations and all have caused delayed germination. Mannose specific lectins appreciated for their efficiency against fungal pathogens. Our results getting an accord with above statement and the lectins were able inhibited the spore germination at all the concentrations. Intact spores observed up to 96 hrs at 100 µg/ml proved the strong interference in germination inhibition. The similar finding was reported by [48] that *Curcuma amarissima* completely inhibited the *invitro* growth of three pathogenic fungi namely *Fusarium oxysporum*, *Exserohilum turicicum*, and *Colectrotrichum cassiicola*, at a concentration of 17.5 to 35 µg. Similarly, the lectin from *Annona muricata* seeds inhibited the growth of *F. oxysporum, F. solani* and *C.musae* [49]. Lectins with the similar characteristics, isolated from the seeds of *Capsicum annuum* inhibited the germination of spores and hyphal growth in *Aspergillus niger, A. flavus, F. solani and F. graminearum* [50]. Delayed spore germination observed in this present study agrees with the findings of [51] where the lectin/agglutinin from the wheat germ (chitin specific), peanut (galactose-specific) and soybean (N-acetyl- D-glucosamine and galactose-specific) were bound to the spores and hyphal tip of both the fungus, *Penicillium* and *Aspergillus* and affected its growth and delayed the spore germination of *A.ochraceus*. The chitin binding lectins, WGA, and potato lectin demonstrated their interference with the chitin synthesis by binding specifically to the hyphal septa of *Trichoderma viride* and *Botrytis cinerea* and inhibited the hyphal growth as well as spore germination respectively [52].

D-galactoside-specific lectin was isolated [53] from the Demosponge, (*Halichondria okadai*) and documented a moderate antifungal activity against some of the phytopathogenic fungus *Botryodiplodia theobromae, Alternaria alternate, Macrophomina phaseolina, Curvularia lunata, Colletotrichum corchori,* and *Fusarium equiseti*. Lectin from the seeds of *Sophoraalope curoides* (SAL) showed inhibition activity *on Penicillium digitatum* and *A. alternata*, at an inhibitory concentration (IC50) of 3.125 and 3.338 mM [54]. Effect of fucose specific lectins of *Aleuria aurantia, Urtica dioica* (UEA-I) and *Anguilla anguilla* against *Mucorra cemosus* was found that AAL, exhibits anti fungal activity where as the UEA-I and AAA failed to do the same. This indicates that the activity of AAL against *Mucorra cemosus* is unique [55]. Spore inhibition assays performed with *Setcreasea purpurea* lectin inhibited the germination of *Rhizoctonia solani, Penicillium italicum, Sclerotinias clerotiorum* and *Helmin thosporiunmaydis* at a minimum concentration of 48.1, 48.1, 96.2 and 96.2 µg/ml, respectively and did not show any effect against *Candida albicans, Aspergillus niger* and *Trichoderma reesei* even up to1.51 mg/ml concentration [56].

As fungi possess thick and rigid cell wall, lectins cannot interact with glycol conjugates of the cell wall. The anti fungal effect of lectins is due to its binding nature to glycoconjugates (glycoproteins / glycolipids) present in the fungal structure. Seed lectin from *Dioclea guianensis* Benth (Dgui) inhibited conidial germination of *C. gloeosporioides* but did not bind to germinated conidia and germ tubes and thereby it was not inhibitory to mycelial growth. The lectins with the same characteristics isolated from *Canavalia ensiformis* (ConA) and *C. maritima* (ConM), had no effect on the fungus, as a conclusion, *C. gloeosporioides* conidia might have surface-specific germination targets recognized by Dgui but not by its homologues, ConM and ConA [57]. Shrinkage of the spores (Fig.6) was noticed with lectin treated spores were looking intact until 96 hrs. Similarly, different types of abnormalities were reported by different studies as the effect of lectin treatments. [58] described some abnormalities in the fungal conidial structure of the plant pathogenic fungus *F. oxysporum*, *Radicis lycopersici* when it was treated with chitosan at 0.1 mg/ml concentration. Examination of ultrathin sections of conidia showed severe damage; binding of *Aleuria aurantia* (AAL) caused morphological changes like abnormal swelling of the hyphal tips and septa, beaded hyphal tips and lysis of the hyphae in Mucorra cemosus (1µM, 72µg/ml). AAL is supposed to bind to polysaccharides of the fungal cell wall or oligosaccharides of fungal glycoproteins in the cell wall and crosslink to it inhibiting the growth of the fungi. Consequently, a polypeptide from *Amaranthus caudatus* seeds, lectins from *Urtica dioica* and hevein from the rubber *Hevea brasiliensis* were assumed to penetrate the fungal cell and reach the plasma membrane and damages the fungal cells.

4. CONCLUSION

The present study and the supporting reports available are concluding that just one specific group of lectin is not effective against pathogens since all the plant lectins groups were effective against pathogens but the level of the effect depends on the structure of lectin and interaction with their target sites. Hence the number of the binding site in lectin and its interactions with a fungal structure that varies among the same group of lectins was confirmed. All the effort on lectins from isolation, purification, and other characterization regards to their functions is to take them as a promising tool against pest and another disease of crops. The choice of a lectin to be used for crop protection must also depend on its non-toxicity towards human being and animals and definitely on the working mechanism. Although Zingiberous lectins have not explored on tea pest and disease control, detailed characterization of their properties and similarity with already available potential lectins will lead them to be considered in this concern.

5. ACKNOWLEDGMENT

We thank National Tea Research Foundation, Kolkata, India for funding the project on ‘Lectin: an alternative biotic stress management system in tea’ under which this work was carried out. Thanks are due to Dr. B. Radhakrishnan, Director for allowing us to carry out all the experiments. We thank Dr. K. N. Chandrashekara, former Senior Biotechnologist, Plant Physiology Division,

for his support in designing the experiments. We express our sincere thanks to laboratory colleagues from our and other departments for their kind support during experiments.

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