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Research Article
Defensive Response of *Corchorus olitorius* in Relation to Infestation of *Anomis sabulifera*

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Abstract
Background and Objective: *Corchorus olitorius*, an important fiber crop in Indian economy, is infested by many pests causes it to evolve protective defense against them. In present era, *Anomis sabulifera* is a major threat to jute agronomy. In this study, the morphological and phyto-chemical defense strategy of jute against *A. sabulifera* was determined. **Materials and Methods:** Jute leaf phenology was studied along with standard biochemical and spectrophotometric procedures for the quantification of phyto-chemicals like saponin, alkaloid, flavonoid, tannin, riboflavin, ascorbic acid, thiamine, soluble sugar, protein, lipid, total moisture and ash content. The results were correlated with the pest incidence. **Results:** Phenological characters were improved throughout the jute cultivation period whereas leaf bio-chemistry showed an altered pattern. After pest menace there was depletion in leaf nutrient content although secondary metabolite concentration was found to be increased following pest attack. **Conclusion:** It was concluded that, *C. olitorius* have no morphological defense strategy against *A. sabulifera* whereas it has evolved itself via phyto-chemical defense mechanisms to protect from pest attack. These findings may be helpful to plan better cultural strategy and pest control programme in near future.

Key words: *Anomis sabulifera*, *Corchorus olitorius*, leaf phenology, phyto-chemistry, defense strategy, jute agronomy

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The success of plants in colonizing and dominating almost every habitat on earth depends on their ability of co-evolution to persist in hostile and erratic conditions of earth[1,2]. This was largely due to the presence of effective resistance mechanism which includes physical, chemical and developmental features[3]. This diversity in plants defense mechanism was evolved through their long lasting struggle for co-existence with the herbivorous animals[4]. Throughout this co-existence host and pest interact in broad spectrum that influences organisms at every level including both basic biochemical events and phenological constituent[5]. Study of host-pest dealings revealed that success of phytophagous insects was resulted from effective neutralization of hosts defense mechanism[1,3]. Some of these interactions are reciprocally beneficial, such as pollination but most of the interactions involved insect dependence on plants for food and equivocally plants defend themselves from herbivorous insects[6]. In fact, the pray-predator association is extensive as because almost every plant species is eaten by at least one insect species[7-9]. Therefore, plants not only have adapted to their morphological resistance properties but a variety of secondary metabolites have also been synthesized in plants due to the selective pressure exerted by phytophagous animals[10,11]. *Anomis sabulifera* (jute semilooper) is a major leaf feeding pest of jute plant that causes severe damage to the host plant[4]. During severe infestation *A. sabulifera* feed up to 90% jute leaf that ultimately leads to 50% fibre yield loss[5]. To fulfill the market demand and use of more and more fertilizers and insecticides, jute pests becomes resistant to stress and become major threat for production yield[12]. Jute plants are devoid of physical resistance like epicuticular wax, resin or thorns. Therefore, jute leaves are easily accessible food for semilooper larvae but heterogeneous infestation pattern was recorded during jute cultivation period[13,14]. Utilization of plant defense mechanism is a key component for IPM programme, which looks for minimizing pest damage through a combination of cultural and biological control (natural enemies and endogenous resistance of host plant) with minimal use of chemical methods[15,16]. The present study was designed to evaluate the morphological and phyto-chemical defense response of jute plant, *Corchorus olitorius* in relation to prevalence of jute semilooper, *Anomis sabulifera* which will explore new facet of host plant resistance system for improvement of pest control programme under IPM model.

MATERIALS AND METHODS

Experimental design: The experiment was conducted at 3 administrative blocks of west Bengal namely Raiganj, Hemtabad and Kaliyaganj in 2016 covering the calendar month of April to August in randomized block design with 3 replications adopting standard cultivation practices. Before sowing N, P_2O_5 and K_2O at the rate of 20 kg ha^{-1} were applied as basal application. The jute seeds were sown in a row spacing of 25 cm in small plots of 4$\times$4 m with a gap of 1 m between each plot. At completely grown condition, plant to plant distance was maintained at 6-8 cm apart after thinning. Samples were collected at 4 different growth stages of jute plant at 30, 60, 90 and 120 DAS (Day after Sowing) for further examination.

Estimation of plant characteristics: Plant height, leaf area, leaf roundness, specific leaf area, leaf thickness, laminar length, laminar width, leaf ratio, leaf perimeter and leaf fresh weight were measured by standard method with necessary modification[17,18].

Estimation of leaf phyto-chemistry

Plant material and sample preparation: Jute leaves were collected from 3 study sites of Uttar Dinajpur district, west Bengal, India. The leaves were first washed in tap water and then in double distilled water to remove dirt. The leaves were then dried at room temperature for 7 days, finely powdered and used for quantitative analysis of different phyto-chemicals.

Quantitative assay of phyto-chemicals: The quantitative assays of different phyto-chemical components such as total protein[19], total lipid[20], total sugar[21], alkaloid[22], flavonoid[23], saponin[24], riboflavin[25], thiamin[26], ascorbic acid[27], phenol[22], moisture and ash content of the collected jute leaves were determined according to standard protocols.

Observation on pest incidence: Three replicated plots (1 m^2) in each of the pre-selected sites were observed for incidence of jute semilooper, *Anomis Sabulifera* throughout the jute growing season in the year 2016. Percentage of plant infested with *A. Sabulifera* larvae was estimated by observing top 8 damaged leaves of each plant[18].

Statistical analysis: All the experiments were performed 3 times and data were represented as Mean$\pm$SD for further statistical analysis. Data were analyzed for correlation coefficient (r) with 5% significance level by using statistical tool kyplot version 2.0 beta 15 (32 bit)[28].

RESULTS

Study on the phenological and phyto-chemical defense of jute plant against phytophagous semilooper insects were done in Uttar Dinajpur district of west Bengal during pre-kharif to kharif season of the year 2016.

Observation on morphological characteristics of jute leaf: Morphological data in Table 1 of the present study showed that nutrition contributing factors as, plant height, leaf area, laminar length and width, leaf perimeter and fresh leaf weight were increased rapidly with time. Whereas, limited changes were observed in leaf thickness (118.23-174.25, 118.56-174.66 and 118.35-174.80 µm), specific leaf area (0.033-0.033, 0.032-0.036 and 0.029-0.036), leaf roundness (40.67-40.00, 39.30-42.00 and 39.50-41.00^0) and leaf ratio (2.74-2.95, 2.67-3.25 and 2.71-3.13) at 3 study sites viz., Raiganj, Hemtabad and Kaliyaganj, respectively.

Observation on phyto-chemical characteristics of jute leaf: Quantitative study in Table 2 of jute leaf extract revealed that concentration of leaf nutrients like total sugar, total protein and total lipid rapidly increased from 30-90 DAS. But at late

Table 1: Morphological characteristics of jute leaf (*C. olitorius*) at different growth stages during 2016

Plant characteristics	Time of observation	Raiganj	Hemtabad	Kaliyaganj
Plant height (cm)	30 DAS	102.00±1.26	102.67±0.95	97.50±1.80
	60 DAS	110.97±1.04	111.40±1.40	102.80±1.53
	90 DAS	174.50±1.05	175.20±1.20	165.33±1.55
	120 DAS	232.67±1.56	238.50±1.40	208.73±1.60
Leaf perimeter (cm)	30 DAS	27.00±0.50	26.60±0.60	26.35±0.69
	60 DAS	28.87±0.75	28.97±0.55	28.70±0.44
	90 DAS	29.13±0.80	30.33±0.37	29.00±0.70
	120 DAS	30.57±0.45	31.20±0.56	29.40±0.45
Leaf area (cm^2)	30 DAS	16.34±0.60	15.87±0.48	15.90±0.45
	60 DAS	17.71±0.40	17.18±0.67	16.80±0.67
	90 DAS	29.91±0.30	39.30±0.55	29.18±0.38
	120 DAS	48.20±0.45	52.50±0.67	31.98±0.55
Specific leaf area	30 DAS	0.033±0.003	0.032±0.006	0.029±0.006
	60 DAS	0.041±0.012	0.039±0.008	0.038±0.006
	90 DAS	0.033±0.007	0.028±0.004	0.036±0.012
	120 DAS	0.033±0.008	0.036±0.007	0.036±0.015
Laminar length (cm)	30 DAS	12.40±1.20	11.70±1.20	11.20±1.40
	60 DAS	13.60±1.22	13.20±1.35	12.93±1.67
	90 DAS	15.43±1.15	14.90±0.90	15.03±1.82
	120 DAS	16.23±1.06	15.70±1.25	15.17±1.25
Laminar width (cm)	30 DAS	4.40±0.05	4.33±0.76	4.30±0.56
	60 DAS	4.30±0.20	4.43±0.70	4.40±0.30
	90 DAS	4.70±0.35	4.73±0.34	4.80±0.75
	120 DAS	5.53±0.56	5.65±0.89	4.90±0.34
Leaf ratio	30 DAS	2.74±0.56	2.67±0.34	2.71±0.67
	60 DAS	3.14±0.23	3.00±0.39	2.98±0.67
	90 DAS	3.28±0.78	3.16±0.75	3.14±0.45
	120 DAS	2.95±0.56	3.25±0.45	3.13±0.56
Leaf thickness (µm)	30 DAS	118.23±7.45	118.56±6.65	118.35±4.56
	60 DAS	125.57±4.70	125.74±6.78	125.17±4.45
	90 DAS	162.52±6.56	162.88±4.76	162.26±3.67
	120 DAS	174.25±4.57	174.66±4.56	174.80±4.56
Leaf roundness	30 DAS	40.67±2.45	39.3±3.46	39.50±4.22
	60 DAS	39.33±1.66	38.67±3.56	38.66±1.55
	90 DAS	40.00±2.56	40.00±1.00	39.33±1.46
	120 DAS	40.00±1.75	42.00±3.00	41.00±1.67
Leaf fresh weight (gm)	30 DAS	0.47±0.02	0.50±0.04	0.47±0.04
	60 DAS	0.72±0.04	0.66±0.02	0.63±0.04
	90 DAS	1.03±0.03	1.08±0.02	1.05±0.02
	120 DAS	1.60±0.04	1.45±0.04	1.14±0.05

Data are presented as Mean±SD

Table 2: Phyto-chemical quantification of jute leaf (*C. olitorius*) at different growth stages during 2016

Phyto-chemicals	Time of observation	Raiganj	Hemtabad	Kaliyaganj
Total sugar (mg g^{-1})	30 DAS	7.63±0.31	7.63±0.23	7.43±0.21
	60 DAS	12.56±0.11	12.56±0.11	12.40±0.17
	90 DAS	13.73±0.15	12.43±0.25	13.73±0.21
	120 DAS	5.06±0.47	5.10±0.20	4.96±0.21
Total protein (mg g^{-1})	30 DAS	5.56±0.11	5.90±0.17	5.63±0.05
	60 DAS	10.70±0.40	10.43±0.40	10.8 ±0.36
	90 DAS	12.60±0.10	12.56±0.35	12.56±0.28
	120 DAS	6.26±0.11	5.73±0.05	5.83±0.05
Total lipid (mg g^{-1})	30 DAS	6.63±0.11	6.50±0.10	5.73±0.05
	60 DAS	11.76±1.09	11.43±1.09	11.40±0.51
	90 DAS	12.43±0.05	12.67±0.11	12.10±0.35
	120 DAS	5.76±0.05	6.20±0.10	6.10±0.55
Alkaloid (mg g^{-1})	30 DAS	13.26±0.49	12.80±0.79	12.20±0.60
	60 DAS	24.33±1.90	23.66±0.60	22.86±0.50
	90 DAS	34.16±0.65	33.10±0.40	34.06±0.80
	120 DAS	47.20±0.62	45.03±0.80	47.10±0.55
Flavonoid (mg g^{-1})	30 DAS	3.23±0.35	3.53±0.35	3.40±0.45
	60 DAS	7.56±0.32	7.13±0.06	7.10±0.20
	90 DAS	12.03±0.49	12.26±0.50	14.20±1.64
	120 DAS	18.63±0.40	18.60±0.55	17.46±0.83
Saponin (mg g^{-1})	30 DAS	5.73±0.32	6.23±0.65	5.80±2.60
	60 DAS	10.70±0.55	10.84±0.21	10.83±0.31
	90 DAS	11.90±0.26	12.10±0.53	12.03±0.49
	120 DAS	15.26±0.97	15.26±0.40	14.90±0.81
Riboflavin (mg g^{-1})	30 DAS	0.11±0.02	0.09±0.01	0.08±0.01
	60 DAS	0.26±0.02	0.22±0.01	0.22±0.01
	90 DAS	0.29±0.04	0.26±0.02	0.26±0.01
	120 DAS	0.15±0.02	0.14±0.01	0.15±0.01
Thiamin (mg g^{-1})	30 DAS	0.07±0.01	0.08±0.01	0.06±0.01
	60 DAS	0.11±0.01	0.12±0.01	0.14±0.01
	90 DAS	0.14±0.01	0.13±0.01	0.15±0.01
	120 DAS	0.08±0.01	0.05±0.01	0.10±0.01
Ascorbic acid (mg g^{-1})	30 DAS	0.75±0.03	0.73±0.02	0.59±0.06
	60 DAS	0.92±0.01	0.88±0.05	0.86±0.03
	90 DAS	0.96±0.01	0.94±0.05	0.90±0.03
	120 DAS	0.67±0.03	0.60±0.02	0.62±0.05
Phenol (mg g^{-1})	30 DAS	7.66±0.25	6.70±0.17	7.80±1.45
	60 DAS	8.46±0.25	7.82±0.28	8.20±0.01
	90 DAS	8.96±00.11	9.40±0.25	8.50±0.04
	120 DAS	13.66±0.15	11.93±0.40	13.60±1.09
Moisture (%)	30 DAS	73.24±2.90	77.25±3.12	80.25±3.72
	60 DAS	76.47±1.65	80.12±2.71	84.27±2.17
	90 DAS	76.72±1.82	80.68±2.87	84.12±2.86
	120 DAS	64.42±0.89	64.35±1.56	67.72±1.56
Ash content (mg g^{-1})	30 DAS	23.14±2.56	23.45±1.02	23.58±0.58
	60 DAS	23.56±2.17	23.87±1.55	23.78±1.21
	90 DAS	23.67±1.65	23.89±0.69	24.17±0.62
	120 DAS	24.37±2.18	24.58±0.92	24.25±0.78

Data are presented as Mean±SD

age period (120 DAS) of jute crop, concentration of those leaf nutrients decreased rapidly. From 30-90 DAS growth phase there was lower concentration of secondary metabolites. Whereas, highest concentration of secondary metabolites was recorded at late growth phase (120 DAS). Although, phyto-chemical estimation showed that leaf moisture and ash content was increased throughout the crop development.

Inter-relationship within leaf phenology and phyto-chemistry: In present study, matrix analysis revealed that major attributing morphological characters in Table 3 were imparted significantly ($p \leq 0.05$) positive effect on each other, except specific leaf area. Table 4 showed a different pattern of inter-dependence between leaf chemical contents. Primary metabolites such as leaf carbohydrates, proteins, lipids, ascorbic acid, riboflavin and thiamin which were main

Table 3: Matrix analysis for inter-relationship of jute leaf phenology

Leaf phenology	Leaf perimeter	Leaf area	Specific leaf area	Petiol length	Petiol thickness	Laminer length	Laminer width	Leaf ratio	Leaf thickness	Leaf roundness	Leaf fresh weight
Leaf perimeter	0.00										
Leaf area	0.87*	0.00									
Specific leaf area	-0.09*	-0.45*	0.00								
Petiol length	0.92*	0.97*	-0.22*	0.00							
Petiol thickness	0.84*	0.86*	-0.57*	0.84*	0.000						
Laminer length	0.95*	0.91*	-0.37*	0.86*	0.84*	0.000					
Laminer width	0.78*	0.98*	-0.52*	0.57*	0.86*	0.83*	0.00				
Leaf ratio	0.55*	0.10*	0.37*	0.78*	-0.57*	0.46*	-0.07	0.00			
Leaf thickness	0.91*	0.94*	-0.48*	0.12^{NS}	0.78*	0.99*	0.87*	0.36*	0.00		
Leaf roundness	0.97*	0.45*	-0.10^{NS}	0.96*	0.15^{NS}	0.37*	0.52*	-0.37*	0.48*	0.00	
Leaf fresh weight	0.93*	0.98*	-0.32*	0.79*	0.96*	0.93*	0.95*	0.22*	0.94*	0.32*	0.00

Significant at *p≤0.05

Table 4: Matrix analysis for inter-relationship study of jute phyto-chemicals

Phyto-chemicals	Sugar	Protein	Lipid	Alkaloid	Flavonoid	Saponin	Riboflavin	Thiamin	Ascorbic acid	Phenol
Sugar	0.000									
Protein	0.982*	0.000								
Lipid	0.995*	0.951*	0.000							
Alkaloid	-0.817*	-0.771*	-0.825*	0.000						
Flavonoid	-0.815*	-0.776*	-0.826*	0.989*	0.000					
Saponin	-0.295	-0.131	-0.219	0.407	0.355	0.000				
Riboflavin	0.951*	0.987*	0.975*	-0.758*	-0.769*	-0.006	0.000			
Thiamin	0.925*	0.971*	0.952*	-0.783*	-0.799*	0.033	0.885*	0.000		
Ascorbic acid	0.931*	0.931*	0.941*	-0.826*	-0.865*	-0.168	0.921*	0.926*	0.000	
Phenol	-0.829*	-0.833*	-0.861*	0.940*	0.961*	0.115	-0.859*	0.900*	-0.906*	0.000

Significant at *p≤0.05

nutrient contributing factors showed significantly (p≤0.05) positive interaction with each other. But interestingly, leaf nutritional characters and secondary metabolites imparted significant (p≤0.05) negative effects between them. Out of the all secondary metabolites phenol exerted highest negative influence on leaf nutrients.

Correlation of pest (*A. sabulifera*) incidence with leaf morphology and phyto-chemistry of jute: Correlation studies Table 5 and 6 showed that relationship of incidence of *A. sabulifera* with the leaf morphology and phyto-chemistry of jute crop that were tested for understanding plants defense against pest herbivory in 2016. The results revealed that among various morphological characteristics, plant height, laminar length, laminar width, leaf perimeter, leaf area, leaf thickness and leaf roundness showed significant positive influence of pest menace. Whereas, specific leaf area, leaf ratio and leaf fresh weight imparted a negative impact on pest incidence.

Correlation analysis also revealed that pest menace had a significant negative impact on leaf nutrient such as sugar (p≤0.05), protein (p≤0.01), lipid (p≤0.01), riboflavin (p≤0.001), thiamine (p≤0.001) and ascorbic acid (p≤0.001). However, pest attack leads to increase in alkaloid (p≤0.01), flavonoid (p≤0.05), saponin (p≤0.05), phenol (p≤0.01), moisture (p≤0.001) and ash content (p≤0.001).

Table 5: Correlation of *A. sabulifera* with jute leaf morphology during 2016

Correlation (r)	Raiganj	Hemtabad	Kaliyaganj
Plant height	0.8440***	0.7525***	0.9184***
Leaf perimeter	0.8983***	0.8896***	0.9242***
Leaf area	0.4826*	0.5132**	0.6326***
Specific leaf area	-0.9080***	-0.8954	-0.9214***
Laminar length	0.3138*	0.3861*	0.3846*
Laminar width	0.8747***	0.8636***	0.8812***
Leaf ratio	-0.8829***	-0.8739***	-0.8983***
Leaf thickness	0.9385***	0.9195***	0.9742***
Leaf roundness	0.9414***	0.9198***	0.9549***
Leaf fresh weight	-0.9036***	-0.8915***	-0.9137***

Significant at *p≤0.05, **≤0.01, ***≤0.001, NS: Not significant

Table 6: Correlation of *A. sabulifera* with phytochemistry of jute leaf during 2016

Correlation (r)	Raiganj	Hemtabad	Kaliyaganj
Sugar	-0.5343*	-0.5515*	-0.5986*
Protein	-0.6228**	-0.6041**	-0.6616**
Lipid	-0.6050**	-0.5816**	-0.6639**
Alkaloid	0.5997**	0.5556**	0.6613**
Flavonoid	0.5425*	0.5613*	0.4477*
Saponin	0.5847*	0.5864*	0.5490*
Riboflavin	-0.9056***	-0.8931***	-0.9195***
Thiamine	-0.9071***	-0.8943***	-0.9204***
Ascorbic acid	-0.9010***	-0.8879***	-0.9153***
Phenol	0.7504**	0.7630**	0.7304**
Moisture	0.9769***	0.9728***	0.9737***
Ash	0.8012***	0.7973***	0.8307***

Significant at *p≤0.05, **≤0.01, ***≤0.001, NS: Not significant

DISCUSSION

The crop morphology mainly the yield attributing characters such as crop height, leaf length, width, perimeter, area and thickness were found increasing towards ripeness of the crop, whereas, specific leaf area, roundness and leaf ratio did not show incremental trend towards maturity stages of plant during study period of 2016. Incidence of jute semilooper showed a positive influence on yield attributing characters of the jute crop. Increase in plant vigour after pest attacks was to compensate the damaged caused by pest herbivory[29,30] as jute plants don't have any strapping physical barrier against its pest. Increased leaf thickness made them more harden to eat by phytophagous pest which was first line of defence against herbivorous pest[14]. These findings were of similar with the result of the study made by Rahman and Khan[18]. However, plant can't defend them only with this morphological defence line and there must be collaboration between physical and chemical[14] defence machineries.

Phyto-chemicals played important role in nutritional ecology and growth of a phyto-phagous insect[3]. Host-plant selection of herbivorous pest was influenced by their capability to ingest and assimilate food matter to transform it into body tissues[31]. According to Roy[32] the development and survival of insects showed significant differences with respect to their food quality. Primary metabolites such as carbohydrate, protein and lipid were key factor for general vitality, growth and reproduction of phytophagous insects[33]. Further, during diapauses lipids provide energy for metabolic processes and also act as precursors of ecdysteroid[34]. Water content in host leaves played an important role in growth rate of plant-fed larvae[18]. On the other hand, secondary metabolites including phenols, flavonoids, terpenoids, alkaloids, etc., concluded the fitness of the food utilization of the phytophagous pest and thus govern plant-insect interaction[14]. Consumption of greater amount of secondary metabolites was found to significantly reduce adult longevity, fecundity and retardation of larval growth[35]. Present study revealed that young and mature leaves (30-90 DAS) of jute plant provide the best quality food for the major defoliator, *A. sabulifera*, because of higher nutritional factors relative to the anti-nutritional secondary metabolites that resulted into high pest incidence. Whereas, secondary metabolites was dominating over leaf nutrients in late growth phase (120 DAS), that limits pest menace. The better understanding of this defence system will allow us to reach more useful methods for the biological control of insect pests with natural products by the development of new jute varieties with enhanced chemical defences.

CONCLUSION

It was concluded that jute crop had not developed any distinct physical barrier in the form of epicuticular wax or resin or thorns. Instead of that, plant vigour attracted its pest initially. On the other hand, pest attack induce host plants chemical defence by elevating different secondary metabolites in the form of phenols, alkaloids, flavonoids and saponin which act as feeding deterrent to eliminate pest population. The stipulated information provides a well decorated knowledge about host-pest interaction and defence strategies of jute crop against *A. sabulifera* that will helpful to generate IPM model for sustainable production of jute crop.

SIGNIFICANCE STATEMENT

Present study revealed the influence of *A. sabulifera* on the defensive strategies of *C. olitorius* which are a major threat to jute cultivation. This study will help the researcher to explore the vital areas of host-pest interaction for jute crop which can be beneficial for construction of improved crop culture practice and pest control.

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