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Biochemical basis of resistance against major castor pests

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ABSTRACT

A field experiment was carried out during Rabi,2021 at RARS, palem to study the biochemical factors responsible for insect resistance. The biochemical parameters viz., crude fibre, crude protein, tannins, phenols and carbohydrates were quantified in five castor hybrids to find out their association with tolerance or resistance against two defoliators Achaea janata and Spodoptera litura and two sucking insects, leafhoppers and thrips. Crude fibre and carbohydrates had a positive correlation with the insect population whereas phenols, tannins and crude protein showed a significant negative correlation. DCH-519 and NBCH-22 with least crude fibre, carbohydrates and higher phenols are considered to be resistant to insect population.

Keywords: *Castor, A.janata, S.litura, leafhopper, thrips, crude fibre, crude protein, tannin, phenol, carbohydrate.*

INTRODUCTION

Castor (*Ricinus communis*) a non-edible oilseed crop widely distributed throughout the tropics and subtropics due to its minimal demand on soil fertility and moderate rainfall requirements (9). It produces only 0.15% of the world's vegetable oil. This oil is the only commercial source of a hydroxylate fattyacid. (10).

India is the largest producer and exporter of castor beans in the world. In 2021-22 the area under castor was 1.484 lakh hectares, out of which 1.022 lakh hectares is in Gujarat followed by Rajasthan with 0.254 lakh hectares and Telangana with 0.036 lakh hectares. According to the government's third advance estimates in 2020-21 total castor production in India is 17.74 lakh tonnes.

One of the major constraints that limits castor productivity is excessive damage caused by castor semilooper, *Achaea janata* L., tobacco caterpillar, *Spodoptera litura* F., and capsule borer, *Conogethes punctiferalis* Guenee, Leafhoppers,*Empoasca flavescens* and Thrips,*Retithrips syriacus*. Semilooper and tobacco caterpillars are active during the vegetative stage, causing defoliation of up to 50%.

Insecticides of various types are effective in lowering pest populations. However, lack of consideration for crop protection principles, as well as the indiscriminate and widespread use of synthetic pesticides, has resulted in issues such as insecticidal resistance, the resurgence of secondary pests, the destruction of natural enemies, pollution, and health risks. To combat pest problems, particularly those involving insects, attempts have been undertaken to find effective and environmentally sustainable alternatives to chemical pesticides (11). One such mechanism is host plant resistance.

Plant resistance as a control method is particularly suited for castor as it is hardy, grown under rainfed conditions on marginal lands as a patch, border or intercrop by small farmers who do not usually resort to any pest control methods. There is no thorough information on how castor germplasm reacts to various insect infestations. The development of suitable resistant/tolerant types is an ideal component that comes at no extra cost, is compatible with other pest control approaches, and is free of pollutants to prevent pest population growth. Plants with a variety of biophysical and biochemical characteristics play a vital role in giving resistance to a variety of insect pests (5).

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MATERIAL AND METHODS

A total of 5 castor genotypes viz., DCH-519, DCH-177, ICH-166, NBCH and PCH-111 were tested for the infestation of castor semilooper, tobacco caterpillar, leafhoppers and thrips at research farm of RARS palem, PJTSAU, Telangana. The experiment was laid out in a Randomized block Design and each treatment was replicated thrice. Plot size of each treatment was 5mX7m(35m^2) with a spacing of 120cmX45cm. All agronomic practices were followed as per the recommendations. No plant protection measures were taken up. Pest infestation was started from 30DAS. Leaf samples of the infested plants of each hybrid were collected at 60DAS.Biochemical parameters viz., crude fibre, crude protein, tannins, phenols and carbohydrates were quantified for different hybrids and correlated with the defoliators population at 60DAS. All the parameters were tested in MFPI-Quality control, PJTSAU, Hyderabad using specific analysis methods.

Crude fiber

Weights of empty fiber bag(M1) were taken and approx. 1g of moisture and fat free dehydrated mango pulp sample(M2) was weighed into the fiber bags. Blank value was determined by placing a blank fiber bag without sample and weight taken as B1. Bags were loaded in the sample carousel which was kept into the glass container. The glass container was placed axial on the previewed position of the hot plate and started the programme in the Fibertherm in which acid and alkali were added at 1.25%. After completion of program, finer bags were removed from the carousel and dried completely in hot air oven at 102^{0}c for 2hrs. Dried fiber bags were kept into crucibles.

Weight of fiber bags and crucible after digestion and drying was M3. For blank value, weight of crucible with blank fiber bag after digestion (B3) was recorded. The crucibles were incinerated in a muffle furnace at 600^{0}c for hrs followed by cooling in a hot air oven for 30 min at 105^{0}c and then cooled in a desiccator and weighed to the nearest 0.1mg(M4). Crucible+blank fiber bag weight after incineration was B4.

% crude fiber=[(M3-M1-M4)-(B3-B1-B4)] x100/M2

Crude protein

Approx 0.2g of standard EDTA was weighed in aluminium foil cups in triplicates. Approx 0.2g of the dehydrated mango pulp was weighed in duplicates. Leico protein analyzer was calibrated with blanks. Standard weights were entered and labeled in the instrument as EDTA and the instrument calibrated by placing them sequentially in triplicates in the crucible. Sample I.D and weights were entered and placed in the crucibles one by one to analyse for protein %

Total phenols

Total phenols were determined using Folin-Cicocalteu reagent. In this method, 0.5ml of the diluted sample reacted with 2.5ml of 0.2mol/L Folin-Cicocalteu reagent for 4 min and then 2ml of saturated sodium carbonate solution was added into the reaction mixture. Absorbance readings were taken at 760nm after incubating at room temperature for 2 hours. The reference standard used is gallic acid and the results are expressed as mg GAE/100gm.

Tannins

Tannin was determined as tannic acid, following a procedure whereby finely ground samples were deflated in diethyl-ether containing 10% acetic acid to 0.2g each of deflated samples, 10ml of 70% aqueous acetone was added. The mixture was shaken for uniform agitation for 2minutes at 31^{o}C at120rev/min and was further agitated for 10mins using vortex mixer and centrifuge at 3.500g for 5mins in a Gallen Kamp (angle head) centrifuge. Exactly 0.2ml of the supernatant was made up to 1ml with 0.5ml folic ciocalteau reagent, 2.5ml of 20% sodium carbonate and distilled water. The colour was allowed to develop for a minimum of 40mins and absorbance was read at 725mm curve of tannic acid in blank and standard were plotted against absorbance. Amount of total phenol as tannic acid equivalent was calculated and expressed on a dry matter basis.

Carbohydrates

Weigh 100mg of the sample into a boiling tube. Hydrolyse by keeping it in boiling water bath for 3 hours with 5mL of 2.5 N-HCl and cool to room temperature. Neutralize it with solid sodium carbonate until the effervescence ceases. Make up the volume to 100ml and centrifuge. Collect the supernatant and take 0.5 and 1ml aliquots for analysis. Prepare the standards by taking 0, 0.2, 0.4, 0.6, 0.8 and 1mL of the working standard. '0' serves as blank. Make up the volume to 1mL in all the tubes including the sample tubes by adding distilled water. Then add 4mL of anthrone reagent. Heat for eight minutes in a boiling water bath. Cool rapidly and read the green to dark green color at 630nm.Draw a standard graph by plotting the concentration of the standard on the *X*-axis versus absorbance on the *Y*-axis. From the graph calculate the amount of carbohydrate present in the sample tube. Carbohydrate present in 100mg of sample =(mg of glucose ÷ Volume of test sample) x100.

RESULTS AND DISCUSSION

The crude fibre ranged between 7.28% and 9.88% at 60DAS. Highest per cent was recorded in PCH-111 followed by DCH-177 and least in NBCH-22.

The crude protein per cent in the hybrids varied from 31.92 to 34.86 with DCH-519 having the highest per cent and PCH-111 having the least crude protein content.

Phenol content (mg GAE/100gm) in the five castor hybrids ranged between 1206.09 (PCH-111) and 1320.24 (DCH-519).

The tannins (mg TAE/100g) ranged between 1.46 and 1.64 at 60DAS. Highest tannin content was recorded in DCH-519&NBCH-22 and least in PCH-111.

The carbohydrates per cent in the castor hybrids varied from 10.7 to 14.7 with PCH-111 and DCH-177 having the highest per cent and DCH-519 having the least carbohydrates content.

Least number of castor semilooper ie, 1.47larva/plant were observed in DCH-519 followed by 1.93 in NBCH-22. Lowest number of *S.litura* ie, 1.02larva/plant was noticed in DCH-519 followed by 1.32 in NBCH-22. DCH-519 was found to better with lowest number of leafhoppers(2.27) and 5.33 thrips/plant.

Correlation studies were carried out between the biochemical parameters and population of major insect pests in castor. Highest pest population was recorded in PCH-111 followed by DCH-177which had higher crude fibre and carbohydrates and lowest population was observed in DCH-519 and NBCH-22 with high phenols, tannins and protein content. Among the five parameters tested protein, phenols and tannins are significantly negatively correlated with the insect population. The negative correlation of phenols and insects is in concurrence with (4) who observed lower incidence of yellow stem borer in rice, due to increased phenolic content. Anti nutritive and defensive properties of phenolic compounds reported by(12)enhanced in castor due to damage by *A.janata* and *S.litura*. The soluble protein content showed significant positive relationship with *S. litura*.at 40 and 60 DAS in soybean(6). The quantities of biochemical constituents ((amino acids, urea, ammonia, carbohydrates, proteins, phenols) were increased by the feeding of leaf eating insects than sucking pests in castor(8). The interaction of *Retithrips syriacus* with the hosts is governed essentially by the biochemical profiles of its hosts, which tend to be altered subsequent to infestation, thus manifesting induced resistance through enhanced production of phenolics (1).The phenols content in the castor leaves had a significant negative correlation with leafhopper population while total carbohydrates present in the leaves had a significant positive correlation with leafhopper population(7). Cotton genotypes possessing more phenols and tannins exhibited resistance or tolerance against leafhoppers was reported by (2) The positive correlation between defoliators and carbohydrates was significant and non significant with fiber. The positive correlation of carbohydrates and defoliators observed was similar to the findings of (14) on shoot and fruit borer incidence on brinjal. The damage index of the three alfalfa cultivars by thrips showed a significant positive association with tannin(13). Tannins act as feeding deterrents against many insects so they may play role in controlling these insects(3).

Conclusion

The crude fibre and carbohydrates in the castor hybrids increases the pest incidence whereas phenols, tannins and crude proteins have a negative effect on the insect population. Higher phenol content makes the plant resistant whereas carbohydrate and fibre makes the plant susceptible to insect

population. So, the genotypes with higher phenols should be grown to reduce the pest population.

Conflict of interest

There is no conflict of interest

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Table. 1. Biochemical parameters and defoliator population on different castor hybrids

S. No	Hybrids	Castor semilooper (Larva/plant)	Tobacco caterpillar (Larva/plant)	Leaf hoppers	Thrips	Crude fibre (%)	Crude protein (%)	Total phenols (mg GAE/ 100g)	Tannins (mg TAE/ 100g)	Carbohydrate (%)
1	ICH-66	2.23	1.89	3.73	5.73	8.97	32.60	1239.11	1.63	12.0
2	DCH-177	3.27	2.80	5.8	6.67	9.79	32.85	1227.31	1.59	14.7
3	PCH-111	3.53	3.00	6.87	7.53	9.88	31.92	1206.09	1.46	14.7
4	DCH-519	1.47	1.02	2.27	5.33	7.81	34.86	1320.24	1.64	10.7
5	NBCH-22	1.93	1.32	4.47	5.8	7.28	34.09	1254.20	1.64	12.1

Table. 2. Correlation and regression equation between biochemical parameters of castor and the incidence of A. janata

Biochemical parameters against *A. janata*	Correlation	Regression
Crude fibre Vs *A. janata*	0.9011	y=5.7809+1.1927x
Crude protein Vs *A. janata*	-0.8679*	y=6.1710-1.1694x
Phenols Vs *A. janata*	-0.8801*	y=6.8847-1.2400x
Tannins Vs *A. janata*	-0.8308*	y=1.7714-0.0722x
Carbohydrates Vs *A. janata*	0.9844*	y=7.8858+1.9928x

**=Significant at 5% level of significance NS=non-significant*

Table. 3. Correlation and regression equation between biochemical parameters of castor and the incidence of S. litura

Biochemical parameters against *S.litura*	Correlation	Regression
Crude fibre Vs *S. litura*	0.9425	y=6.2285+1.2549x
Crude protein Vs *S. litura*	-0.8946*	y=5.6961-1.2124x
Phenols Vs *S. litura*	-0.8721*	y=6.8594-1.1063x
Tannins Vs *S. litura*	-0.8093*	y=1.7347-0.0707x
Carbohydrates Vs *S. litura*	0.9648*	y=8.8988+1.9647x

**=Significant at 5% level of significance NS=non-significant*

Table. 4. Correlation and regression equation between biochemical parameters of castor and the incidence of leaf hoppers

Biochemical parameters against Leafhoppers	correlation	Regression
Crude Fibre	0.7189^{NS}	6.739+0.4693x
Crude protein	-0.7995*	5.7227-0.5313x
Total phenols	-0.9083*	8.2538-2.0103x
Tannins	-0.8269*	1.7559-0.0354x
Carbohydrates	0.9605*	8.4016+0.9590x

**=Significant at 5% level of significance NS=non-significant*

Table. 5. Correlation and regression equation between biochemical parameters of castor and the incidence of Thrips

Biochemical parameters against Thrips	correlation	Regression
Crude Fibre	0.7980^{NS}	2.2013+1.0535x
Crude protein	-0.8064^{*}	3.9966-1.0838x
Total phenols	-0.8267*	7.0949-4.5191x
Tannins	-0.9423*	2.0992-0.0816x
Carbohydrates	0.9344*	1.1176+1.8870x

**=Significant at 5% level of significance NS=non-significant*

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