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Effects of leaf and root bark of *Calotropis procera* and *Parquetina nigrescens* on protein profile of *callosobruchus maculatus* (coleoptera: chrysomelidae)

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Abstract
This study assessed the effects of leaf and root bark extracts of *Calotropis procera* and *Parquetina nigrescens* on the protein profile of *Callosobruchus maculatus.* These were with a view to developing a new biological control measure of the insect pest at molecular level. The SDS-PAGE patterns of adult male and female *C. maculatus* that emerged from 4th instar larvae treated with 0.5% methanolic extract of leaf and root bark of *C. procera* and *P. nigrescens*, including untreated male and female and the four instar larvae and pupa were determined. The result shows that the leaf and root bark extract of *C. procera* and *P. nigrescens* influenced changes in protein profile of *C. maculatus*. The extracts of *C. procera* leaf and *P. nigrescens* root bark caused an increase in the number of protein fractions of adult male and female *C. maculatus* as against decrease observed with the extracts of *C. procera* root bark and *P. nigrescens* leaf.

Keywords: molecular weight fractions, instar larvae

Introduction
Electrophoresis of proteins has been successfully used for the characterization of different evolutionary, taxonomic and genetic relationship studies (Ladizinsky and Van, 1984) [1].

It separates mixtures of proteins based on charge, charge to mass ratio, size, or shape. This technique is mainly used as an analytical and preparative tool, especially one-dimensional separation, often employed as a pre-fractionating technique (Jorgenson and Evans, 2004) [2]. Often, laboratories use one-dimensional gel electrophoresis to evaluate the outcome of protein purification preceding the analysis by two-dimensional gel electrophoresis (Chen *et al.,* 2007) [3]. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) technique is a tool for estimating the molecular weights of proteins (Weber *et al.,* 1971) [4]. It exploits differences in molecular size to resolve difference in protein fractions as little as 1% in their electrophoretic relative mobility through the gel matrix (Scopes, 1994) [5]. A major advantage of electrophoresis over morphological evaluation is the ability to analyze for genetic variations on molecular basis (Srivastava and Gupta, 2002) [6].

In line with electrophoretic method, Kudupali and Shivanna (2013) [7] reported the quantitative analysis of male accessory gland secretory proteins of 11 species of Drosophila analyzed using SDS-PAGE technique with the molecular weights of the proteins, ranging from 12-134 kDa.

Materials and Methods
Insect Treatment
The following levels of insect treatments were used for the protein electrophoretic analysis

- Adult male of *C. maculatus* from 4th instar larvae treated with 0.5% methanolic leaf extract of *C. procera*.
- Adult female of *C. maculatus* from 4th instar larvae treated with 0.5% methanolic leaf extract of *C. procera*.
- Adult male of *C. maculatus* from 4th instar larvae treated with 0.5% methanolic root bark extract of *C. procera*.
- Adult female of *C. maculatus* from 4th instar larvae treated with 0.5% methanolic root bark extract of *C. procera.*
- Standard
- Adult male of *C. maculatus* from 4th instar larvae treated with 0.5% methanolic leaf extract of *P. nigrescens*
- Adult female of *C. maculatus* from 4th instar larvae treated with 0.5% methanolic leaf extract of *P. nigrescens*
- Adult maleof *C. maculatus* from 4th instar larvae treated with 0.5% methanolic root bark extract of *P. nigrescens*
- Adult female emergence of *C. maculatus* from 4th instar larvae treated with 0.5% methanolic root bark extract of *P. nigrescens*
- Untreated adult maleof *C. maculatus*
- Untreated adult female of *C. maculatus*
- Untreated first instar larvae of *C. maculatus*
- Untreated second instar larvae of *C. maculatus*
- Untreated third instar larvae of *C. maculatus*
- Untreated fourth instar larvae of *C.maculatus*
- Untreated pupae of *C. maculatus.*

Specimen preparation for protein analysis
0.1 g of insect was homogenized with 1 mL of 0.85% saline solution. This was stored in eppendorf tube at room temperature for 2 hours. The homogenate was centrifuged

using microcentrifuge at 10,000 g. Supernatant was collected and defatted using 200 µl of toluene at a time until the sample was free of fat substance. The protein extract was stored on ice until use.

Preparation of gels

Protein profile was carried out as described by Smith (1976). Separating gel of 10% was composed of 4ml of deionized water, 2.5 mL of Tris buffer (1.5M, pH 8.8) (by dissolving 18.15 g Tris Base in 80 ml of distilled water, pH was adjusted using 6 N HCl), 3.33 ml of acrylamide, 100 µl of 10% sodium dodecyl sulfate (SDS) 50 µl of 10% ammonium persulfate (APS), and 15 µl of N, N,N',N'-tetramethylethylene diamine (TEMED) and stacking gel of 4% contained 3.35 mL of deionized water, 2.5 mL of Tris buffer (0.5 M, pH 6.8) (by dissolving 6 g Tris Base in 80 ml of distilled water, pH was adjusted using 6 N HCl), 4 mL of acrylamide, 100 µl of 10% SDS, 50 µl of 10% ammonium persulfate (APS), and 15 µl of N, N,N',N'-tetramethylethylene diamine (TEMED). The prepared separating gel was poured in between the plates leaving 0.5 to 1 cm.for stacking gel. This was overlaid with 70% ethanol to a depth of a few millimeters and the gel was allowed to polymerize for 20 minutes. After the separating gel has polymerized, the ethanol was rinsed from the surface with distilled water and excess water drained. The stacking gel mix was added and. appropriate combs were inserted. This was allowed to polymerize for 30 minutes.

Loading and running of samples

An aliquot of 10 µl of the sample were diluted with 30 µl of sample buffer (1 mL of electrophoresis buffer, 3 mL of glycerol, 0.2 mL of 0.5% Bromophenol and 5.8 mL of distilled water) and heated at 94^0C for 4 minutes and cooled inside the freezer for 1 h. 10 µl of the samples were loaded into the gel for running with running buffer added at 150 milivolts for 1 hour after the combs were removed. After running of the gel, the gel was removed and stained in Coomassie stain solution foe 1 h and destained in destaining solution of 50% methanol,
10% acetic acid and 40% distilled water.

Gel Analysis

Gel Analyzer (2010) was used to analyze the electrophoresis gel.

Result

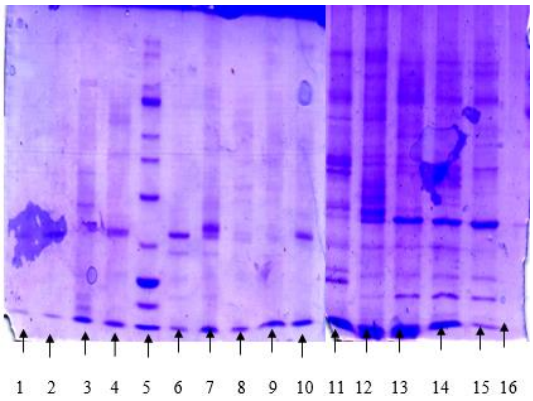

Fig 1: The SDS-PAGE patterns of adult male and female *C.maculates* that emerged from 4th instar larvae treated with 0.5% methanolic extract of leaf and root bark of *C. procera* and *P. nigrescens*, including untreated male and female and the four instar larvae and pupa.

Legend: (1) Adult male of *C. maculatus* from 4th instar larvae treated with 0.5% methanolic leaf extractof *C. procera*; (2) Adult female of *C. maculatus* from 4th instar larvae treated with 0.5% methanolic leaf extractof *C. procera*; (3) Adult male of *C. maculatus* from 4th instar larvae treated with 0.5% methanolic root bark extract of *C. procera*; (4) Adult female of *C. maculatus* from 4th instar larvae treated with 0.5% methanolic root bark extract of *C. procera;* (5) Standard; (6) Adult male of *C. maculatus* from 4th instar larvae treated with 0.5% methanolic leaf extract of *P. nigrescens;* (7) Adult female of *C. maculatus* from 4th instar larvae treated with 0.5% methanolic leaf extract of *P. nigrescens;* (8).Adult male of *C. maculatus* from 4th instar larvae treated with 0.5% methanolic root bark extract of *P. nigrescens;* (9) Adult female emergence of *C. maculatus* from 4th instar larvae treated with 0.5% methanolic root bark extract of *P. nigrescens;* (10) Untreated adult male of *C. maculatus;* (11) Untreated adult female of *C. maculatus;* (12) Untreated first instar larvae of *C. maculatus;* (13) Untreated second instar larvae of *C. maculatus;* (14) Untreated third instar larvae of *C. maculatus;* (15) Untreated fourth instar larvae of *C.maculatus;* (16) Untreated pupae of *C. maculatus*

Table 1: Molecular weight fractions of male and female adult *C. maculatus* that emerged from 4th instar larvae treated with 0.5% methanolic leaf extract of *C. procera*

	Treated	male		Untreated	Male		Treated	Female		Untreated	female
Lane	Band	Molecular Weight (kDa)	Lane	Band	Molecular Weight (kDa)	Lane	Band	Molecular Weight (kDa)	Lane	Band	Molecular Weight (kDa)
1	1	163	10	1	187	2	1	146	11	1	199
1	2	134	10	2	129	2	2	135	11	2	169
1	3	117	10	3	103	2	3	99	11	3	124
1	4	96	10	4	82	2	4	77	11	4	99
1	5	88	10	5	72	2	5	71	11	5	78
1	6	71	10	6	66	2	6	57	11	6	64
1	7	50	10	7	50	2	7	46	11	7	56
1	8	46	10	8	46	2	8	38	11	8	43
1	9	38	10	9	44	2	9	32	11	9	39
1	10	26	10	10	38	2	10	23	11	10	29
1	11	23	10	11	33	2	11	18	11	11	22
1	12	21	10	12	27	2	12	16	11	12	19
1	13	18	10	13	23	2	13	15	11	13	18
1	14	16	10	14	20	2	14	14	11	14	15

1	15	15	10	15	17				11	15	14
1	16	14	10	16	14						
1	17	14	10	17	13						
1	18	13									

Table 2: Molecular weight fractions of adult male and female of *C. maculatus* that emerged from 4th instar larvae treated with 0.5% methanolic root bark extract of *C. procera*.

	Treated	male		Untreated	Male		Treated	Female		Untreated	female
Lane	Band	Molecular weight (kDa)	Lane	Band	Molecular weight (kDa)	Lane	Band	Molecular weight (kDa)	Lane	Band	Molecular weight (kDa)
3	1	196	10	1	187	4	1	146	11	1	199
3	2	135	10	2	129	4	2	110	11	2	169
3	3	112	10	3	103	4	3	77	11	3	124
3	4	86	10	4	82	4	4	67	11	4	99
3	5	67	10	5	72	4	5	62	11	5	78
3	6	61	10	6	66	4	6	45	11	6	64
3	7	53	10	7	50	4	7	38	11	7	56
3	8	44	10	8	46	4	8	31	11	8	43
3	9	38	10	9	44	4	9	24	11	9	39
3	10	29	10	10	38	4	10	18	11	10	29
3	11	25	10	11	33	4	11	15	11	11	22
3	12	18	10	12	27	4	12	14	11	12	19
3	13	16	10	13	23				11	13	18
3	14	15	10	14	20				11	14	15
3	15	14	10	15	17				11	15	14
			10	16	14						
			10	17	13						

Table 3: Molecular weight fractions of male and female adults of *C. maculatus* that emerged from 4th instar larvae treated with 0.5% methanolic leaf extract of *P. nigrescens*

	Treated	male		Untreated	Male		Treated	Female		Untreated	female
Lane	Band	Molecular Weight (kDa)	Lane	Band	Molecular Weight (kDa)	Lane	Band	Molecular Weight (kDa)	Lane	Band	Molecular Weight (kDa)
6	1	199	10	1	187	7	1	191	11	1	199
6	2	101	10	2	129	7	2	108	11	2	169
6	3	86	10	3	103	7	3	82	11	3	124
6	4	71	10	4	82	7	4	76	11	4	99
6	5	60	10	5	72	7	5	62	11	5	78
6	6	44	10	6	66	7	6	47	11	6	64
6	7	39	10	7	50	7	7	33	11	7	56
6	8	29	10	8	46	7	8	29	11	8	43
6	9	26	10	9	44	7	9	24	11	9	39
6	10	23	10	10	38	7	10	20	11	10	29
6	11	20	10	11	33	7	11	17	11	11	22
6	12	17	10	12	27	7	12	16	11	12	19
6	13	16	10	13	23	7	13	15	11	13	18
6	14	14	10	14	20	7	14	14	11	14	15
			10	15	17				11	15	14
			10	16	14						
			10	17	13						

Table 4: Molecular weight fractions of adult male and female *C. maculatus* that emerged from 4th instar larvae treated with 0.5% methanolic root bark extract of *P. nigrescens*

	Treated	male		Untreated	Male		Treated	Female		Untreated	female
Lane	Band	Molecular Weight (kDa)	Lane	Band	Molecular Weight (kDa)	Lane	Band	Molecular Weight (kDa)	Lane	Band	Molecular Weight (kDa)
8	1	194	10	1	187	9	1	189	11	1	199
8	2	101	10	2	129	9	2	157	11	2	169
8	3	76	10	3	103	9	3	120	11	3	124
8	4	66	10	4	82	9	4	103	11	4	99
8	5	56	10	5	72	9	5	75	11	5	78
8	6	47	10	6	66	9	6	59	11	6	64
8	7	43	10	7	50	9	7	53	11	7	56
8	8	34	10	8	46	9	8	44	11	8	43
8	9	31	10	9	44	9	9	42	11	9	39
8	10	23	10	10	38	9	10	33	11	10	29

8	11	20	10	11	33	9	11	30	11	11	22
8	12	18	10	12	27	9	12	24	11	12	19
8	13	15	10	13	23	9	13	23	11	13	18
8	14	14	10	14	20	9	14	20	11	14	15
			10	15	17	9	15	18	11	15	14
			10	16	14	9	16	16			
			10	17	13	9	17	14			

Table 5: Molecular weight fractions of untreated first, second, third, fourth instar larvae and pupae of *C. maculatus*

	Ist			2nd			3rd			4th			Pupae	
Lane	Band	Molecular Weight (kDa)	Lane	Band	Molecular Weight (kDa)	Lane	Band	Molecular Weight (kDa)	Lane	Band	Molecular Weight (kDa)	Lane	Band	Molecular Weight (kDa)
12	1	191	13	1	196	14	1	196	15	1	196	16	1	153
12	2	157	13	2	178	14	2	175	15	2	161	16	2	117
12	3	121	13	3	137	14	3	155	15	3	134	16	3	103
12	4	96	13	4	110	14	4	123	15	4	109	16	4	81
12	5	76	13	5	93	14	5	110	15	5	99	16	5	58
12	6	63	13	6	76	14	6	98	15	6	76	16	6	39
12	7	52	13	7	59	14	7	77	15	7	64	16	7	37
12	8	48	13	8	54	14	8	58	15	8	58	16	8	33
12	9	44	13	9	44	14	9	53	15	9	53	16	9	26
12	10	41	13	10	41	14	10	43	15	10	49	16	10	22
12	11	34	13	11	38	14	11	40	15	11	39	16	11	20
12	12	31	13	12	34	14	12	31	15	12	31	16	12	16
12	13	28	13	13	28	14	13	26	15	13	29	16	13	14
12	14	26	13	14	26	14	14	22	15	14	25			
12	15	22	13	15	22	14	15	18	15	15	23			
12	16	18	13	16	18	14	16	16	15	16	21			
12	17	16	13	17	16	14	17	14	15	17	18			
12	18	14	13	18	15				15	18	16			
			13	19	14				15	19	14			

Fig. 1 and Table 1 shows the electrophoresis and molecular weight fractions of male and female adults of *C. maculatus* reared from 4th instar larvae exposed to cowpea seeds treated with 0.5% methanolic leaf extracts of *C. procera.* There were 19 protein fractions in the treated male ranging between 13 to 163 kDa while there were 14 in treated females ranging from 14 to 146 kDa. Generally, males that emerged from treated larvae have higher molecular weight fractions than the females when their protein fractions were compared. Molecular weight fractions of protein band one (163 kDa) of the male was higher than corresponding female band (146 kDa). Protein band 2 for male and female were 134 kDa and 135 kDa respectively. Other protein fractions not present in females were 13, 21, 26, 50, 88, 96 and 117 kDa. There were 17 molecular weight fractions in the untreated males ranging between 13 and 189 kDa with minor variations from the treated males. However, a molecular weight fraction 187 kDa is specific to untreated males. Molecular weight fractions of untreated females ranged from 14 to 199 kDa with 15 bands. Molecular weight fractions 124, 169, and 199 kDa were specific to untreated females. There were minor variations in the molecular weight fractions of treated and untreated females with higher molecular weight fractions in the untreated females than the treated females.

Table 2 represents the molecular weight fractions of adult male and female *C. maculatus* reared from 4th instar larvae on exposed to cowpea seeds treated with root bark methanolic exract of *C. procera*. Males treated with extracts had 15 protein fractions that ranged from 14 to 196 kDa while the female had 12 protein fractions ranging between 14 and 146 kDa. Males from treated larvae had higher number of protein bands than females similarly treated when their protein bands were compared. All the protein bands in treated males had higher molecular weight fractions than those of females. Molecular weight fractions of 16, 25, 29, 44, 53, 61, 86, and 112 kDa were specific to treated males while 24, 31, 45, 62, 77 and 110 kDa were specific to treated females. Molecular weight fractions were higher in the untreated than the treated males and females of *C. maculatus.*

Table 3 shows the molecular weight fractions of adult male and female *C. maculatus* that emerged from 4th instar larvae treated with 0.5% methanolic leaf extract of *P. nigrescens.* There were 14 protein bands in male and female with molecular weight fractions ranging between 14 to 199 kDa and between 14 to 191 kDa respectively. In all the different molecular weight fractions between treated males and females, protein band 14 with molecular weight fraction of 14 kDa was similar for both sexes. Molecular weight fractions 23, 26, 39, 44, 60, 71, 86, and 101 kDa were specific for treated males while fractions 15, 24, 33, 47, 62, 76, 82, 108 and 191 kDa were specific for treated females. Untreated males had more protein bands than the treated males. Similarly, untreated female had one more protein bands than treated female. Adult male and female from treated larvae had the same number of band while untreated male had more protein fractions than untreated female.

Molecular weight fractions of adult male and female *C. maculatus* reared from 4th instar larvae exposed to cowpea treated with 0.5% methanolic root bark extract of *P. nigrescens* are as shown in Table 4. Fourteen protein fractions were present in the treated males ranging from 14 to 194 kDa while the untreated males had 17 protein fractions that ranged from 13 to187 kDa. Females from treated larvae had 17 protein fractions ranging between 14

and 189 kDa. However, 15 protein fractions in the untreated females ranged from 14 to 199 kDa. Molecular weight fractions of protein band two for treated males (101 kDa) was lower than the corresponding treated females (157 kDa). Most of the molecular weight fractions present in the treated males were not present in the treated females while those that were present in the females were absent in the males.

Table 5 shows the molecular weight fractions of untreated first, second, third, fourth instar larvae and pupae of *C. maculates*. There were 18 protein fractions in the untreated first instar larvae ranging from 14 to 191 kDa while untreated second, third and fourth instar larvae had 19, 17, and 19 protein fractions respectively with their molecular weight fractions ranging from 14 to 196 kDa. Similarities in the molecular weight fractions (14, 16, and 18 kDa) were observed in all the four instars larvae and included 50% of low molecular weight fractions. However, 7 specific molecular weight fractions (48, 52, 63, 96, 121, 157, and 191 kDa) were present in the first instar larvae while 15, 38, 54, 59, 93, 137, and 175 kDa were unigue to second instar larvae. The third instar larvae had specific molecular weight fractions 40, 43, 77, 98, 123, 155, and 175 kDa while the fourth had 21, 23, 25, 29, 39, 49, 64, 99, 109, 134, and 161 kDa. There were 13 protein bands present in the untreated pupae of *C. maculatus* with their molecular weight ranging from 14 to 153 kDa. The molecular weight fractions of 14 and 16 kDa present in pupal stage were also present in all the four larval stages. First and second instar larvae shared the same molecular weight fractions of 14, 16, 22, and 26 kDa with pupa while third and fourth instar larvae shared 14, 16, 22. 26, and 58 kDa and 14, 16, 39, and 58 kDa with pupa respectively. However, specific molecular weight fractions observed in pupal stage when compared with first, second, third and fourth instar larval stages included; 20, 33, 37, 81, 103, 117, and 153 kDa.

Discussion

The molecular weight fractions of *C. maculatus* that emerged from 4th instar larvae treated with methanolic leaf and root bark extracts of *C. procera and P. nigrescens*, larvae of first to fourth instar and pupae of *C. maculatus* revealed differences in protein synthesis (Saini and Sarin, 2012) [8] and from larval to pupal stage, the number of protein bands comparatively reduced (Choi *et al.,* 2004) [9]. Plant extract may be responsible for the inhibition of protein synthesis by forming a protein complex (El-Sherhaby *et al*., 2008) [10]. Insecticidal activity of the plant extracts also may reduce feeding efficiency and protein content of an insect´s body which may account for low protein fractions recorded in treated insects as observed in this study (Etebari *et al.,* 2007) [11]. The range of molecular weight fractions as observed in this study is closely related to the report of Kudupali and Shivanna (2013) [7] who worked on 11 species of *Drosophila.* It could be suggested from this study that interaction between *C. maculatus* and plant extracts may lead to physiological and biochemical changes ((Saini and Sarin, 2012) [8]. The extracts of *C. procera* leaf and *P. nigrescens* root bark caused an increase in the number of protein fractions of adult male and female *C. maculatus* as against decrease as observed with the extracts of *C. procera* root bark and *P. nigrescens* leaf, hence, high concentration of the extracts of *C. procera* root bark and *P. nigrescens* leaf could be used to control *C. maculatus* at molecular level.

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