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RESEARCH ARTICLE

BIOCHEMICAL BASIS OF COWPEA RESISTANCE TO BRUCHID, *CALLOSOBRUCHUS MACULATUS* (F.).

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Abstract

Bruchids are the most destructive pest of stored cowpea seeds leading to losses in quantity and quality of seeds. To overcome this problem, farmers use different synthetic insecticides but these have toxic effects on the environment and non-target organisms. The use of resistant genotypes is considered a cost effective and safe alternative to synthetic insecticides. The metabolites are reported to play a major role in resistance but the basis of this role in resistance to the bruchid is not well understood. Understanding the biochemical mechanisms of pest resistance could be utilized in exploiting the trait in crop breeding. The present study investigated the roles of seed coat (condensed and free tannins, flavonoids, total phenolic content and their anti-oxidant activity) and cotyledon (carbohydrate, proteins and α-amylase inhibitory activity) biochemical compounds in conferring cowpea seed resistance to bruchid infestation. None of the seed coat biochemical traits were associated with the seed resistance parameters. With the exception of protein content which was only associated with weight loss, all the cotyledon biochemical traits were strongly associated with all of the seed resistance parameters. These results indicated that seed coat biochemical traits have no role in conferring resistance but cotyledon biochemical traits namely, α-amylase inhibitory and carbohydrate content are involved in conferring resistance to bruchid attack by reducing the growth and development of the pest. These traits can be used as biochemical markers for quick and accurate selection of cowpea genotypes resistant to bruchid.

Introduction:-

Cowpea (*Vigna unguiculata (L.) Walp*.) is the second most important and widely consumed grain legume in semi-arid and subtropical regions of Africa (NRC, 2006). It is cultivated primarily as a pulse, vegetable and as cover and fodder crop (Faye, 2005). Cowpea seeds provide a rich source of protein, carbohydrate, minerals and vitamins, particularly for the poorer sectors in many developing countries (Gonçalves et al., 2016; Hamid et al., 2016). In Uganda, cowpea is the fourth most important legume crop after beans, groundnut and soybean (Ddungu et al., 2014). It is grown by approximately 2.2 million smallholder farmers on total area of 77,000 ha (FAOSTAT, 2011)

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but the crop's production is limited by a variety of constraints including insect pests, diseases and parasitic striga (Terao et al., 1997). In storage, cowpea bruchid (*Callosobruchus maculatus*) is the most destructive pest (Deshpande et al., 2011) resulting in partial or total stored crop losses (NARO, 2012; Joel and Hyuha, 2010).

More than 63% of cowpea growing farmers in northern Uganda use synthetic pesticides to minimize losses due to bruchids (MBAZARDI, 2014). However, the use of synthetic insecticides to control bruchid infestation has widespread environmental contamination, toxicity to non-target organisms and negative effects on human health (Isman, 2008). The growing concerns about the harmful effects of pesticides have stimulated investigations in new, safer alternative pest control strategies, including the use of resistant genotypes (Castro et al., 2013; Tripathi et al., 2015). An understanding of the mechanisms of resistance to the bruchid is one of the key pre-requisites for designing effective and efficient strategies to breed resistant genotypes. Many studies have indicated that chemical compounds, such as phenolic acids, tannins, and flavonoids and α-amylase inhibitor may confer cowpea seed resistance to *C. maculatus* (Ojwang et al., 2012). For example, Lattanzio et al. (2005) reported elevated level of α-amylase inhibitor as the main mechanism for resistance to bruchid. Gatehouse et al. (1979) reported that elevated level of trypsin inhibitor in TVu 2027 cowpea seeds was responsible for resistance to *C. maculatus*. In contrast, Baker et al. (1989) found no significant correlations between the levels of trypsin inhibitor and *C. maculatus* development time or mortality. There are also conflicting reports on the effect of seed coat tannin content on the oviposition and survival of *C. maculatus*. For instance, Lale and Makoshi (2000) reported a positive association whereas Lattanzio et al. (2005) found no significant association between seed coat tannin content and seed resistance to bruchid. Also, Edde and Amatobi (2003), in their experiments using 22 cowpea varieties found no significant differences in number of eggs deposited, adult mortality and mean developmental periods between cowpea with and without seed coat, suggesting that seed coat had no value in protecting cowpea seeds against attack by *C. maculatus*, rather the resistance factors were carried in the cotyledon and embryo of the seed. Thus, it seems that cowpea seeds do not rely on one type of chemical defense, implying that resistance might be due to the accumulation of several biochemicals. However, direct evidence of the protective roles of these compounds against bruchid is limited. In view of this, the aim of the present study was to examine the influence of biochemical attributes including protein, carbohydrate, flavonoid and tannins content, phenolic compound and their anti-oxidant activity; and α-amylase inhibitory activities in three resistant and four susceptible cowpea genotypes on *C. maculatus* infestation.

Materials and methods:-

Sources of germplasms:-

Seven cowpea genotypes differing in response to bruchid infestations used in this study (Table 1) were identified out of 145 cowpea genotypes by a no-choice laboratory bruchid damage bioassay (Miesho *et al.* unpublished manuscript). All the genotypes except TVU-2027 were obtained from the Germplasm Maintenance Project of Makerere University Agricultural Research Institute, Uganda.

Experimental design and data collection:-

The experiment was laid in a completely randomized design (CRD) with three replicates per genotype. For each replicate, data were collected on biochemical parameters namely, free and condensed tannins, phenolic content and their antioxidant activities, flavonoids, carbohydrates, proteins and α- inhibitor activity.

Extraction and quantification of biochemical parameters:-

Prior to biochemical analyses, the seeds were soaked in phosphate buffered saline for one day and dehulled manually.

Seed coat biochemical analysis and anti-oxidant assay of extracted phenolics

The seed coat biochemical attributes were assessed by refluxing defatted seed coat flour in 80% hexane using a soxlet apparatus. Total phenolics, flavonoids, free and condensed tannins were extracted, quantified and recorded.

Total phenolic and total flavonoids

Total phenolic content was extracted with 80% aqueous methanol containing 1% HCl (1:50, w/v) by refluxing in a boiling water bath for 30 minutes. The refluxed material was concentrated under vacuum in a rotary flash evaporator (RU 10 C SO99, IKA, Germany) and used for determining the total phenolic content (TPC) and total flavonoid content (TFC). The TPC of each extract was determined using the method described by Chandrasekara and Shahidi (2010) and the contents expressed in mg of gallic acid equivalents (GAE) per gram of defatted flour. The TFC of

flour was measured by the aluminium chloride colorimetric assay method described by Kim et al. (2003) by reading the absorbance at 510 nm (Biowave ii+, Cambridge, England) and the contents expressed in percent of gallic acid equivalents (GAE) per gram of defatted flour.

Free and condensed tannins

To determine free and condensed tannins, about 0.1g of cowpea defatted seed coat flour was placed in a 1.5ml Eppendorf tube and 0.5ml acetone (70%): ascorbic acid (1%) solution was added. The solution was shaken for 20 minutes using an orbital shaker (Unimax 1010 DT, Germany). Thereafter, petroleum ether (0.5ml) was added and the solvent left to evaporate. 0.3ml of distilled water was then added and the sample centrifuged at 10 rpm for 10 min. An aliquot of 0.1ml was taken and 0.4ml of HCl-butanol solution (5%v/v) added. The tube was placed in a water bath at 80^0C for 70 minutes. Absorbance was then read at 550 nm to quantify the amount of free tannins. For condensed tannins, the remaining solution was drained from the tubes, and 0.2ml distilled water and 0.8ml of acid-butanol solution were added and the tube placed in a water bath (80^oC) for 70 minutes. Absorbance, which is directly proportional to the tannin content, was read at 550 nm (Biowave ii+, Cambridge, England). The contents were expressed in mg of tannic acid (TA) per gram of defatted flour.

Ferric ion-reducing capacity assay (FRC)

The extracted phenolics were assayed for their antioxidant activities following the method of Pownall et al. (2010), with slight modifications. Briefly, various sample dilutions (500 µL) in 50 mM phosphate buffer (pH 7.0) were mixed with 250 µL of 1% (w/v) potassium ferricyanide solution and incubated for 20 min at 50ºC. Thereafter, 500 µL of 10% (v/v) trichloroacetic acid was added and centrifuged at 3000 r/minute for 10 minutes. The supernatant was mixed (500 µL) with 100µL of 0.1% (w/v) ferric chloride (freshly prepared) and 500 µL of distilled water followed by additional incubation for 10 min, absorbance was immediately measured at 700 nm (Biowave ii+, Cambridge, England) against a blank consisting of phosphate buffer and the appropriate volume of solvent, treated in the same manner. The results were expressed as absorbance units at 700 nm, which was considered as a measure of reducing power. Higher absorbance of the reaction mixture indicated greater reducing power.

Cotyledon biochemical analysis and α-amylase inhibitory assay

Total protein assay

Total protein was quantified using the Bradford method (Bradford, 1976). A sample of 0.1g of cotyledon flour was added into a falcon tube containing 5ml of distilled water. The solution was agitated for 30 minutes at 50^oC on a thermomixer (Eppendorf AG, Hamburg, Germany). From this protein solution, 0.1 ml was pipetted off and stained with 3 ml of Bradford reagent (Commassie brilliant blue + 95% Ethanol + 85% Phosphoric acid + Deionized water). The stained proteins were quantified by measuring absorbance in a spectrophotometer (Biowave ii^+, Cambridge, England) at a wave length of 595nm against standard albumin.

Total carbohydrate

Total carbohydrate was determined as total starch and sugar content by hydrolysis of 0.1g sample with 5ml of 10% (v/v) Sulphuric acid at 80^oC in a waterbath (Grant TXF 200, England) for 30 minutes. The sample was left to cool to room temperature and the resultant sugar as well as the original sugar was quantified using the method of Dubois et al. (1956). 0.5 ml of solution was diluted with 1ml of deionized water and dehydrated using 1ml of concentrated sulphuric acid. The resulting furfural compound was estimated by adding 0.5ml of 5% phenol and the resulting colored compounds quantified by measuring its absorbance at a wavelength of 490 nm in a spectrophotometer (Biowave ii^+, Cambridge-England) against a starch soluble standard.

The α-amylase inhibitors assay

To determine the α-amylase inhibitory activity, ten grams of finely ground de-hulled seeds were incubated in an eppendorf thermomixer (Eppendorf AG, Hamburg, Germany) at 200 rpm with 0.15M NaCl (1:5, w/v), followed by centrifugation at 12100 × *g* for 60min. The supernatant was buffered by adding 0.2M Na-succinate, 0.1M CaCl2 (pH 3.8) (110 µlml−1) and was heated in a water bath at 70^oC for 15min. The protein precipitate was removed by centrifugation (12100 × *g* for 60min) and the clear supernatant was brought to pH 5.6 with NaOH. Ethanol, (19% final concentration) was added to this solution and the mixture was stirred for 3.5h at 4^oC and then centrifuged (Moreno et al., 1990). The protein inhibitory activity of *α*-amylase inhibitor extracts was determined using the Bradford method using bovine serum albumin as a standard (Bradford, 1976). A sample of 10µl of extracted α-amylase inhibitors was added to 50µl of standard *α*-amylase enzyme (extracted from *Aspergillus Oryzea* and supplied from SIGMA) in a total volume of 1.2 ml of barbital buffer solution, pH 6.5. The mixture was incubated at

37°C for 10 minutes followed by the addition of 0.2ml of substrate solution (0.1% potato starch solution in water). After incubation at 20°C for 10 min, the reaction was stopped with 0.2ml 3M HCl. The undigested starch was determined by adding 0.4ml of potassium iodide (I2–KI) solution (1.2 and 1.8mM, respectively) and by measuring the change in absorbance at 620 nm. Controls without inhibitors were included to determine the amylase activity of each preparation (expressed as amylase units, i.e., the amount of enzyme that gave 50% hydrolysis of the added starch) (Silano et al., 1975). The α-amylase inhibitory activity (percentage of control) was expressed as a percentage of the α-amylase activity values in the absence of pre-incubation with the seed extract.

Statistical analysis

A one way analysis of variance (ANOVA) was used to test differences in biochemical characteristics among cowpea genotypes. Least significant difference (LSD) test was used to separate means. Pearson correlation was used to examine the association between each biochemical characteristic and seed resistance to bruchid parameters (number of eggs, median development period, growth index, average number of holes, percentage weight loss, percentage pest tolerance and Dobie susceptibility) for the studied genotypes. Multiple linear regression was used to identify the relative significance of each biochemical attributes in determining the seed resistance. All analyses were conducted using GenStat Discovery, 16th Edition statistical package.

RESULTS:-

Cowpea genotype biochemical resistance characteristics

There were significant differences ($P< 0.001$) in the seed nutritional (total protein and total carbohydrate) and anti-nutritional factors (free tannins, condensed tannins, phenolic compounds and their ferric ion-reducing capacity assay (FRC), flavonoids and α- amylase inhibitor activity) among the cowpea genotypes (Table 2).

Cowpea seed nutritional contents and their effect to C. maculatus

The studied cowpea genotypes varied significantly ($P< 0.001$) in their seed nutritional contents (Table 3). High total protein content was recorded from susceptible genotype IT109 (29.0%) whereas the lowest was obtained from another susceptible genotype WC69 (19.4%). The highest total carbohydrate content was recorded from resistant genotype WC42 (69.5%) and the lowest from susceptible genotype IT71 (59.0 %) (Fig. 1 B).

Cowpea seed anti-nutritional contents and their effect on C. maculatus

The studied cowpea genotypes showed significant differences in their seed anti-nutritional contents (Table 3). There were more condensed tannins and phenolic compounds in seeds than free tannins and flavonoids. The mean condensed tannins and phenolic compounds were 4.70mgTA/g and 17.02mgGAE/g, respectively. The mean condensed tannin content was about four times higher than that of free tannins (1.33mgTA/g). Susceptible genotype WC69 (7.62mgTA/g) had the highest amount of condensed tannins whereas the lowest (2.91mgTA/g) was recorded also from susceptible genotype IT71. The amount of free tannins ranged from 1.10mgTA/g (susceptible, WC69) to 2.19mgTA/g in susceptible genotype SECOW2W. Flavonoids ranged from 0.26%GAE in resistant genotype 2419 to 1.83%GAE in resistant genotype WC42. The highest phenolic compound was recorded from resistant genotype WC42 (38.48mgGAE/g) and the lowest from resistant genotype TVu 2027 (11.09mgGAE/g). The highest Ferric ion-reducing capacity assay (FRC) was recorded from susceptible genotype WC69 (76.76) and the lowest again from susceptible genotype IT109 (22.74). Highest α-amylase inhibitory activity was recorded on resistant genotype 2419 (13.356µl) and the lowest was recorded from susceptible genotype WC69 (44.97µl) (Fig 1A).

Relationship among cowpea biochemical attributes and resistance parameters to bruchid:-

The extent to which the studied traits contributed to increase resistance to bruchid was given by information obtained through correlation studies and multiple regression analysis. Total carbohydrate content was positively and significantly ($P<0.001$) correlated to median development period ($r = 0.82$) and pest tolerance ($r = 0.79$); and negatively correlated to percentage weight loss ($r = -0.85$), number of eggs ($r = -0.72$), average number of holes ($r = -0.78$), insect growth index ($r = -0.79$) and Dobie susceptibility index ($r = -0.79$). The α-amylase inhibitory activity was positively and significantly ($P<0.001$) correlated to the percentage weight loss ($r = 0.81$), number of eggs ($r = 0.75$), average number of holes ($r = 0.80$), insect growth index ($r = 0.71$) and Dobie susceptibility index ($r = 0.78$); and negatively correlated to median development period ($r = -0.55$) and percentage pest tolerance ($r = 0.80$) (Table 4). The multiple linear regression analysis showed that 87.7% of the total variability in Dobie Susceptibility could be predicted using α-amylase inhibitory activity, total protein and total carbohydrate content of the seeds (Table 5).

Discussion:-

Results of the study confirm the importance of certain biochemical attributes for resistance against cowpea bruchids. Among the biochemical attributes, only total carbohydrate content and α-amylase inhibitory activity of the seeds were associated with resistance to bruchid (Table 4). Although all the analyzed genotypes contained varied quantities of proteins, condensed and free tannins, flavonoids and phenolic compounds and ferric ion-reducing capacity assay, none of them was associated to seed resistance to bruchid (Table 4). For example, cowpea line TVu 2027, an accession classified as bruchid-resistant line showed low concentration of condensed and free tannins whereas WC69 and SECOW2W, the susceptible lines contained very high concentrations of condensed and free tannins. Additionally, the highest and lowest flavonoid contents were recorded only from resistant genotypes WC42 and 2419, respectively. Likewise, the highest phenolic compounds and ferric ion-reducing capacity were recorded from resistant genotypes WC42 and TVu 2027, respectively (Table 3). The lack of correlations between all of the individual seed coat biochemical attributes and the seed resistance parameters found in this study showed that seed coat biochemical attributes were not effective barriers against *C. maculatus*. Similar results were reported by Baker et al. (1989) and Lattanzio et al. (2005). Also, Edde and Amatobi (2003) found no significant correlation between numbers of eggs deposited, adult mortality and mean development periods on cowpea seeds with and without seed coat. These findings, as well as our study suggest that the seed coat may not be a useful aspect to consider for breeding of resistant cowpea varieties to bruchid, rather the resistance factors are carried in the cotyledon and embryo of the seed.

Additionally, the results of the study provides strong evidence that elevated level of α-amylase inhibitory activity in the cotyledons of cowpea genotypes is responsible for conferring resistance of cowpea genotypes to *C. maculatus* (Fig. 1 A). Previous work by Lattanzio et al. (2005) also found a positive relationship between Dobie susceptibility index and the level of α-amylase inhibitory activity suggesting the existence of negative effect of α-amylase inhibitory on bruchid growth and development. Also, α-amylase inhibitory activity has been shown to prolong insect developmental period, cause reduction in the levels of adult emergence and retardation in insect growth by inhibiting enzymes responsible for starch digestion resulting in carbohydrate starvation (Macedo et al., 2004). Amongst the studied cowpeas, the inhibitory activity in 2419, a genotype showing a resistance to the pest, was found to be about three times higher than in WC69, a susceptible genotype (Fig. 1A). When the amount of α-amylase inhibitor was considered, resistant genotypes 2419 and WC42 needed 13.35 and 17.04 µl of α-amylase inhibitors to inhibit 50% of the insect α-amylase enzyme, respectively. On the other hand, susceptible genotypes IT71 and WC69 needed 33.28 and 44.97µl of α-amylase inhibitor. The results also showed that, amongst the different genotypes of the same resistance level, a great variability in the inhibitory activity could be detected, indicating the existence of different types of α-amylase inhibitor in the tested genotypes (Franco et al., 2002). Lattanzio et al. (2005) on cowpea and Wisessing et al. (2010) on mungbeans had reported α -amylase inhibitory activity as the main factor of seed defense against bruchid infestation.

The higher total carbohydrate content in seeds of resistant genotypes compared to those of susceptible ones (Fig. 1 B) is an indication that carbohydrate content could also offer seed defense against bruchid infestation. Carbohydrate content increases resistance by increasing seed hardness (Ajeigbe et al., 2008) thereby making seed penetration by the insect difficult. Furthermore, greater roles of carbohydrate in imparting seed resistance to bruchid damage was fully reflected by its strong correlation with seed resistance parameters and dobie susceptibility index, a measure of resistance to bruchid damage (Dobie, 1974) (Table 4).

Multiple regression analysis results also confirmed the role of α-amylase inhibitory and seed carbohydrate content in conferring resistance to bruchid. The negative correlation relationship between α-amylase inhibitory and median development period, one component of DSI, indicated that prolonging insect development period might be due to α-amylase inhibition. Similarly, the negative relationship between carbohydrate content and median development period also indicates that carbohydrate is responsible for prolonging insect development period in addition to offering physical barrier. On the other hand, the negative association between seed carbohydrate content and α-amylase inhibitory may be an indication that the two traits are controlled by different, overlapping, linked genetic loci (Acquaah, 2012). This information could guide breeders on how to improve resistance in cowpea genotypes by focusing on elevating the level of seed α-amylase inhibitory and carbohydrate content. The regression and correlation results indicate that the α-amylase inhibitory and seed carbohydrate content should be considered when selecting resistant genotypes to bruchid since they had strong correlation and higher contributions to variation in genotypes for resistance to bruchid attack (Table 5).

Table 1:- Cowpea genotypes used in the study (data from Miesho et al. Unpublished manuscript)

Genotypes	NE	MDP (days)	GI	ANH	PWL	PPT (%)	DSI	Resistance status	Cultivar type	Origin
IT109	124	21.5	2.92	7.8	27.6	0	8.8	Susceptible	Improved	IITA
SECOW2W	87.3	22.8	3.92	7.7	24.2	0	8.3	Susceptible	Improved	Uganda
WC69	141	23	2.42	7.7	35.9	0	8.2	Susceptible	Landrace	Uganda
IT71	87	22.8	3.56	6.9	44.7	0	8.1	Susceptible	Inbred line	IITA
WC42	17.3	32	0.23	0.1	0.5	90	0.3	Resistant	Landrace	Uganda
TVu 2027	7	42	0.37	0.1	0	93.3	0.2	Resistant	Improved	Nigeria
2419	39.7	42	0.03	0	0	96.7	0	Resistant	Landrace	Uganda

NE: Number of eggs, MDP: Median development period, GI: Growth index ANH: Average number of holes, PWL: percentage weight loss; PPT: percentage pest tolerance and DSI: Dobie susceptibility

Table 2:- Results of one-way analysis of variance for differences in seed biochemical traits among the studied cowpea

Source of variation	df			Mean squares of traits					
		CT	FT	TFC	TPC	FRC	Protein	Carb	α-AIs
Genotype	6	11.4^{***}	0.44^{***}	1.07^{***}	278.03^{***}	1498.08^{***}	86.68^{***}	43.84^{***}	360.93^{***}
Residual	14	0.06	0.00	0.00	1.70	0.06	0.44	0.31	7.76

CT; Condensed tannins, FT: Free tannins, TFC: Total flavonoid content, TPC: Total phenolic compounds, FRC: Ferric ion-reducing capacity assay of the extracted phenolic compounds, Carb: Carbohydrate, and α-AIs: α-amylase inhibitory activity. *** P<0.001

Table 3:- Estimates of seed biochemical constituents in resistant and susceptible cowpea genotypes to bruchid.

Genotype	CT mg TA/g	FT mg TA/g	TFC %GAE	TPC mg GAE/g	FRC	Protein %	Carb %	α-AIs (µl)
IT109	4.71	1.18	0.67	15.04	22.74	29.0	61.9	24.59
SECOW2W	4.37	2.19	0.83	13.21	27.56	28.4	63.5	32.53
WC69	7.62	1.10	1.80	13.32	76.76	19.4	64.3	44.97
IT71	2.91	1.28	1.14	12.42	35.61	26.2	59.0	33.28
WC42	7.10	1.16	1.83	38.48	67.42	22.2	69.5	17.04
TVU 2027	3.03	1.14	0.70	11.09	27.02	27.5	65.5	20.79
2419	3.16	1.27	0.26	15.57	33.12	26.9	65.0	13.35
LSD	0.45	0.10	0.09	2.28	0.44	1.2	1.0	4.88

CT; Condensed tannins, FT: Free tannins, TFC: Total flavonoid content, TPC: Total phenolic compounds, FRC: Ferric ion-reducing capacity assay of the extracted phenolic compounds, Carb: Carbohydrate, and α-AIs: α-amylase inhibitory activity.

Table 4:- Correlation between cowpea seed biochemical traits with phenotypic bruchid resistance parameters.

	CT	FT	TFC	TPC	FRC	Protein	carb	α-AIs
CT	1							
FT	-0.23^{ns}	1						
FLNDS	0.90^{***}	-0.23^{ns}	1					
TPC	0.36^{ns}	-0.19^{ns}	0.52^{**}	1				
FRC	0.90^{***}	-0.37^{ns}	0.89^{***}	0.40^{*}	1			
Protein	-0.21^{ns}	0.24^{ns}	-0.47^{*}	-0.43^{*}	-0.39^{*}	1		
carb	0.28^{ns}	-0.29^{ns}	0.30^{ns}	0.21^{ns}	0.39^{ns}	0.36^{ns}	1	
amylase	0.35^{ns}	0.20^{ns}	0.42^{*}	-0.29^{ns}	0.29^{ns}	-0.46^{*}	-0.48^{**}	1
PWL_	0.09^{ns}	0.19^{ns}	0.14^{ns}	-0.17^{ns}	-0.04^{ns}	-0.55^{**}	-0.85^{***}	0.81^{***}
NE	0.32^{ns}	0.11^{ns}	0.16^{ns}	-0.35^{ns}	0.11^{ns}	-0.20^{ns}	-0.72^{***}	0.75^{***}
MDP	-0.22^{ns}	-0.35^{ns}	-0.07^{ns}	0.06^{ns}	-0.01^{ns}	0.24^{ns}	0.82^{***}	-0.55^{**}
ANH	0.16^{ns}	0.38^{ns}	0.11^{ns}	-0.32^{ns}	-0.08^{ns}	-0.23^{ns}	-0.78^{***}	0.80^{***}
PPT	-0.15^{ns}	-0.37^{ns}	-0.12^{ns}	0.27^{ns}	0.09^{ns}	0.27^{ns}	0.79^{***}	-0.80^{***}

GI_value	-0.03^{ns}	0.57**	-0.00^{ns}	-0.25^{ns}	-0.27^{ns}	-0.21^{ns}	-0.79**	0.71***
DSI	0.14^{ns}	0.36^{ns}	0.09^{ns}	-0.29^{ns}	-0.11^{ns}	-0.23^{ns}	-0.79***	0.78***

Ns = non-significant; values with *, ** and *** implies significant at *P* = .05, *P* < .01 and P < .001, respectively

Table 5:- Results of multiple regression analysis for the relationship between the biochemical attributes of cowpea seeds and Dobie susceptibility index.

Parameter	**Regression coefficient (b)**	**Adjusted R-square**	***P-value***
α-amylase inhibitory activity	0.24	87.72	0.000
Total protein	0.22		0.008
Total carbohydrate	-0.67		0.000

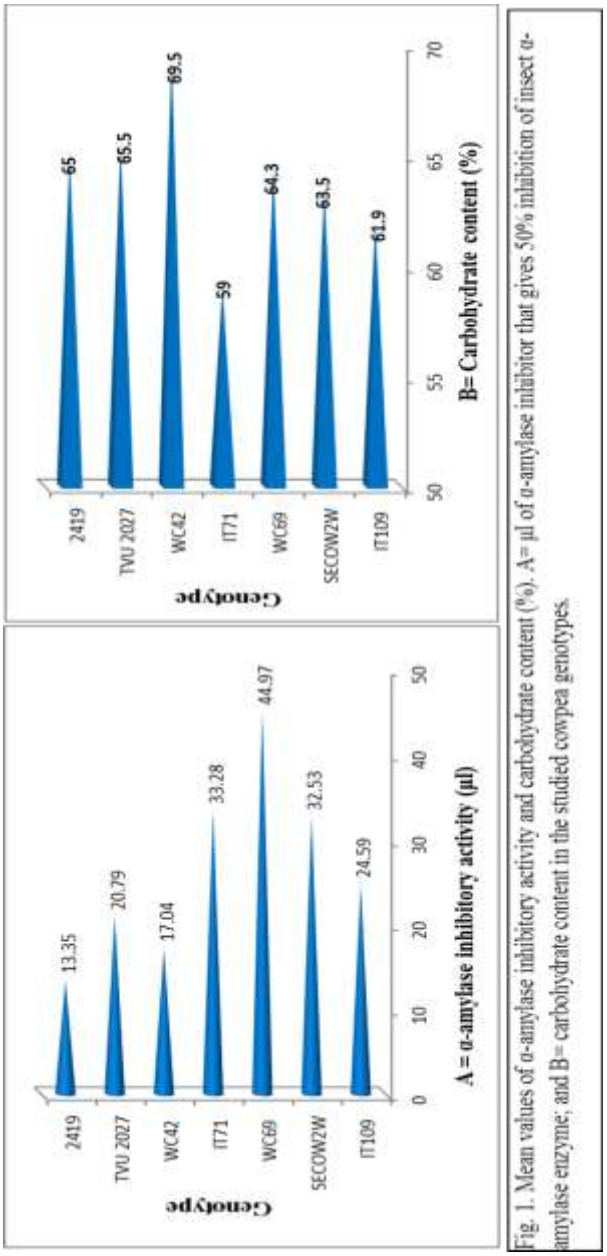

Fig. 1. Mean values of α-amylase inhibitory activity and carbohydrate content (%). A= µl of α-amylase inhibitor that gives 50% inhibition of insect α-amylase enzyme; and B= carbohydrate content in the studied cowpea genotypes.

Conclusion:-

This study provides evidence that the biochemical traits, particularly α-amylase inhibitor and carbohydrate content are responsible for cowpea resistance to *C. maculatus*. These traits were strongly associated with all seed resistance parameters and resistance was indicated by reduced insect population growth and prolonged insect development period. Future studies need to investigate the mode of inheritance of the biochemical traits to guide breeders in introgressing these factors into adapted but susceptible cowpea varieties.

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References:-

1. Acquaah G. (2012): Principles of Plant Genetics and Breeding. 2nd Edition. John Wiley and Sons Inc., Chichester, UK.
2. Ajeigbe H. A., Ihedioha D. and Chikoye D. (2008): Variation in physico-chemical properties of seed of selected improved varieties of Cowpea as it relates to industrial utilization of the crop. *African Journal of Biotechnology* 7, 3642-3647.
3. Baker T.A., Nielsen S.S., Shade R.E. and Singh B.B. (1989): Physical and chemical attributes of cowpea lines resistant and susceptible to *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae). *Journal of Stored Product Research* 25, 1–8.
4. Bradford M.M. (1976): A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye-binding. *Analytical Biochemistry* 72, 248–254.
5. Castro M. J. P., Baldin E. L. L., Cruz, P.L., Zouza C.M. and Silva P.H.S. (2013): Characterization of cowpea genotype resistance to Callosobruchus maculatus. *Pesquisa Agropecuária Brasileira* 48, 1201-1209.
6. Chandrasekara A. and Shahidi F. (2010): Content of insoluble bound phenolics in millets and their contribution to antioxidant capacity. *Journal of Agricultural and Food Chemistry* 58, 6706–6714.
7. Ddungu W. S. P., Ekere J., Bisikwa R. Kawooya D., Kalule O. and Biruma M. (2014): Marketing and market integration of cowpea (*Vigna unguiculata* L. Walp) in Uganda. *Journal of Development and Agricultural Economics* 7, 1-11.
8. Deshpande V.K., Makanur B., Deshpande S.K., Adiger S. and Salimath P.M. (2011): Quantitative and Qualitative losses caused by Callosobruchus maculates in Cowpea during Seed Storage. *Plant Archives* 11, 723-731.
9. Dobie, P. (1974): The laboratory assessment of the inherent susceptibility of maize varieties to post harvest infestations by *Sitophilus zeamais* Mots. (Coleoptera: Curculionidae). *Journal of Stored Products Research* 10, 183-197.
10. Dubois M., Gilles K.A., Hamilton J.K., Rebers P.A. and Smith F. (1956): Colorimetric method for determination of sugars and related substances. *Analytical Chemistry* 28, 350-356.
11. Edde P.A. and Amatobi C.I. (2003): Seed coat has no value in protecting cowpea seed against attack by *Callosobruchus maculatus* (F.). *Journal of Stored Products Research* 39, 1–10.
12. Faye M.D. (2005): Investigations of key aspects for the successful marketing of cowpeas in Senegal. Published Ph.D thesis Department of Agricultural Economics University of the Free State Bloemfontein South Africa.
13. FAO, FAOSTAT (2011): Available online at: http://faostat.fao.org/site/291/default.aspx, Food and Agriculture Organization.
14. Franco O. L., Rigden D. J., Melo F. R. and Grossi-de-Sá M.F. (2002): Plant α-amylase inhibitors and their interaction with insect α-amylases. *European Journal of Biochemistry* 269, 397-412.
15. Gatehouse A.M.R, Gatehouse J.A, Dobie P, Kilminster A.M. and Boulter D. (1979): Biochemical basis of insect resistance in *Vigna unguiculata*. *Journal of the Science of Food and Agriculture* 30, 948–958.
16. Gonçalves A., Goufo P., Barros A., Domínguez-Perles R., Trindade H., Rosa E.A.S., Ferreira L. and Rodrigues M. (2016): Cowpea (*Vigna unguiculata* L. Walp), a renewed multipurpose crop for a more sustainable agri-food system: nutritional advantages and constraints. *Journal of the Science of Food and Agriculture* 96, 2941–2951.

17. Isman B. M. (2008): Perspective Botanical insecticides: for richer, for poorer. *Pest Management Science* 64, 8–11.
18. Mundua, J. (2010): Estimation of Consumer Preferences for Cowpea Varieties in Kumi and Soroti Districts, Uganda. Unpublished MSc. Thesis, Makerere University, Uganda.
19. Kim D., Jeong, S., and Lee, C. Y. (2003): Antioxidant capacity of phenolic phytochemicals from various cultivars of plums. *Food Chemistry* 81, 321–326.
20. Lattanzio V., Terzano R., Cicco N., Cardinali A., Venere D. Di, & Linsalata, V. (2005): Seed coat tannins and bruchid resistance in stored cowpea seeds. *Journal of the Science of Food and Agriculture 85*, 839–846.
21. Macedo M.L.R., De Sa, C.M., Freire M.D.G.M. and Parra, J.R.P. (2004): A Kunitz- type inhibitor of coleopteran proteases, isolated from *Adenanthera pavonina* L. seeds and its effect on *Callosobruchus maculatus*. *Journal of Agricultural and Food Chemistry* 52, 2533–2540.
22. Mbarara Zonal Agricultural Research and Development InstituteI (MBAZARDI). (2014): NarrativeReport, (13).
23. Moreno J., Altabella T. and Chrispeels M.J. (1990) Characterization of α- amylase inhibitor, a lectin-like protein in the seeds of Phaseolus vulgaris. Plant Physiology 92, 703–709.
24. National Agricultural Research Organization (NARO) (2012): Agriculture in Uganda Volum II Crops.
25. National Research Council (NRC) (2006): Lost Crops of Africa'. Vegetables, Volume II: The National Academies Press, Washington, D.C.
26. Ojwang, L.O., L. Dykes and Awika J.M. (2012): Ultra performance liquid chromatography-tandem quadrupole mass spectrometry profiling of anthocyanins and flavonols in cowpea (Vigna unguiculata) of varying genotypes. *Journal of Agricultural and Food Chemistry* 60, 3735-3744.
27. Pownall T.L., Udenigwe C.C. and Aluko R.E. (2010): Amino acid composition and antioxidant properties of pea seed (Pisum sativum L.) enzymatic protein hydrolysate fractions. *Journal of Agricultural and Food Chemistry* 58, 4712-4718.
28. Saima H., Sabeera M., Idrees Ahmed W., Farooq Ahmad M., Mohd B. and Munaf (2016): Physical and cooking characteristics of two cowpea cultivars grown in temperate Indian climate. *Journal of the Saudi Society of Agricultural Sciences* 15, 127–134.
29. Silano V., Furia M., Gianfreda L., Macri A., Palescandolo R., Rab A., Scardi V., Stella E. and Valfre F. (1975): Inhibition of amylases from different origins by albumins from the wheat kernel. *Biochimica et Biophysica Acta* 391, 170–178.
30. Tripathi K. (2012): Differential reaction of cowpea genotypes to pulse beetle under artificial seed infestation and biochemical basis of resistance. Published MSc. thesis Indian Agricultural Research Institute, New Delhi.
31. Tripathi K., Chauhan S. K., Padmawati G. G., Prasad T.V., Srinivasan K. and Bhalla S. (2015): Screening of cowpea [*Vigna unguiculata* (L.) Walp.] accessions against pulse- beetle, Callosobruchus chinensis (L.). *Legume Research* 38, 2015: 675-680.
32. Wisessing A., Engkagul A., Wongpiyasatid A. and Choowongkomon K. (2010): Biochemical Characterization of the α-Amylase Inhibitor in Mungbeans and Its Application in Inhibiting the Growth of Callosobruchus maculatus. *Journal of Agricultural and Food Chemistry* 58, 2131–2137.