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Effect of Nitrogen Fertilizer Rates on Cowpea Growth, Yield and Seed Chemical Properties in Relation to Insect Infestation by *Callosobruchus maculatus*

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Abstract: Nitrogen (N) is essential to crop yield improvement and it can change crops ability to defend against insect pest attacks both in field and postharvest. Cowpea seeds in storage are significantly affected by the pest *Callosobruchus maculatus*. This pest can damage about 50% of the seeds after only fewer months in storage. The aim of the study was to evaluate four rates of nitrogen fertilizer (0.0, 16.5, 33.0 and 66.0 kg N fed^{-1}) on the growth and yield performance of cowpea variety Kafr El-Sheikh-1 as well as some physical and chemical properties of cowpea seeds viz N content %, crude protein content %, P %, K %, total carbohydrate %, crude fiber %, fat content and ash % in relation to infestation with cowpea beetle (*Callosobruchus maculatus*) postharvest through determining susceptibility, egg oviposition, egg hatchability (%), number of adult emergence (F_1) and preferability to rates of nitrogen fertilizer. Two bioassays choice and non-choice were carried out under laboratory conditions. Two experiments were conducted in randomized complete block design with four replicates at the plantation site of El-Balasy village, Sidi Salem Directorate, Kafr El-Sheikh Governorate, Egypt and Stored Product Research Department at Sakha Agricultural Research Station during two successive summer seasons of 2020 and 2021. Almost vegetative growth, seed yield, its components and seed chemical properties of cowpea were significantly increased with increasing nitrogen fertilizer rates up to 66.0 kg N fed^{-1}, except seed total carbohydrate andcrude fiber contents were significantly decreased during both seasons. The differences between nitrogen fertilizer rates of 33.0 and 66.0 kg N fed^{-1} on almost cowpea traits under study were not significant. Results obtained revealed that the all parameters revealed that the biology and weight loss of *C. maculatus* were significantly affected by the different levels of nitrogen fertilizer. The F_1 progeny increased from 15.50 to 42.30 adult with levels of 0.0 and 66.0 kg N fed^{-1}, respectively. Moreover, the cowpea beetle preferred seeds received 33.0 N kg fed^{-1} than that received 0.0 and 66.0 N kg fed^{-1}.

Key words: Cowpea seeds · Nitrogen fertilizer · Seed yield · Seed properties · *Callosobruchus maculatus*

INTRODUCTION

Cowpea [*Vigna unguiculata* (L.) Walp.] is of major importance to livelihoods of relatively poor people in less developed countries of the tropics, especially where animal protein is not easily available for the family Agbogidi [1], El Naim and Jabereldar [2], El Naim *et al.* [3] and Adeyemi *et al.* [4]. It is an important crop in the agriculture of African countries for the following reasons: (1) provision of nutritious food, all the parts of cowpea used for food (fresh leaves, immature pods and the seeds) are nutritious, providing protein, total carbohydrate, vitamins and minerals. The seed contains 22-23 % protein (as opposed to 2 % in cassava and 10 % in maize) and good quantity of thiamine (vitamin B1), riboflavin (vitamin B2) and niacin (vitamin B_3) and richer a significant source of β-carotene and ascorbic acid (vitamin C). Cowpea seeds used as food, supplement very well the protein deficiency of the predominantly total carbohydrate, cereal, root and plantain diet of African communities. A well-

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known problem of cowpea seeds is its content of tannins, trypsin inhibitors and flatulent sugar, raffinose, which cause bloating of stomach when a meal containing cowpea is consumed. As a result, a meal of cowpea is repulsive to some people. This problem can easily be avoided by seed soaking before dehulling, heat treatment and breeding for cowpea varieties without or with low content of these factors Ngalamu *et al.* [5]. In addition to, (2) provision of high quality feed for animals, (3) cowpea as cover crop, (4) provision of organic matter to the soil, (5) fixes atmospheric nitrogen and adds it to the soil about of 40-80 kg N ha^{-1}, (6) drought tolerant or drought hardy, (7) provision of cash, (8) good growth and yield under irrigation in hot tropical dry season and (9) seeds cook quickly Ngalamu *et al.* [5] and Madukwe *et al.* [6].

Nitrogen is important nutrients for cowpea production especially when nitrogen deficient in soils Madukwe *et al.* [6]. Several investigations reported that increasing nitrogen fertilizer rates led to significant increases in plant height and No. of branches $plant^{-1}$ Abayomi*et al.* [7] and Shaban *et al.* [8], No. of pods $plant^{-1}$ and pod length Azarpour *et al.* [9] and Moursi *et al.* [10], No. of seeds pod^{-1} and seed yield $plant^{-1}$ Shaban *et al.* [8] and Singh *et al.* [11], seed index and seed yield fed^{-1} Sebetha *et al.* [12] and Mohammad and Mukhtar [13], seed nitrogen and crude protein contents Adeyemi *et al.* [4] and Mukhtar *et al.* [14], seed phosphorus and potassium contents Moursi *et al.* [10] and Amujoyegbe and Alofe [15] in addition to seed fat and ash contents Musa *et al.* [16] and Daramy *et al.* [17] of cowpea. El-Atawy and Kasem [18] and Muneta *et al.* [19] indicated that mean values ofseed total carbohydrate and crude fiber contents were significantly decreased with increasing nitrogen fertilizer rates.

The green revolution initiated in the mind 1960 and characterized by the successful breeding and widespread adoption of new high yield varieties, pesticides and nitrogen fertilizers has doubled the production of many crops, such as rice, wheat and maize Conway [20]. Cowpea, *Vigna unguiculata* (L.) (Walp) is widely cultivated in the tropics and suptropics as food for man and livestock. The crop is highly prone to pests and diseases which limit its production Baidoo and Mochiah [21]. Cowpea is one of the most important legume crops cultivated by many resources - poor farmers in many countries of tropical Africa, Asia and South America Kabululu [22]. It is a rich source of protein and certain minerals necessary for the healthy growth of humans and animals as cattle Uzogara and Ofunya [23]. According to Bressani [24] the average cowpea contains 23-25% protein, 50-67% carbohydrate and 1.9% fat, making it one of the most nutritious crops. Apart from its importance in the diets of many people in developing countries, it is an important source of hay for cattle in many parts of the world Timko *et al.* [25]. Apart from its importance as a source of food for man and cattle, the crop also contributes to improving and maintaining the fertility of the soil, wherever it is cultivated. Cowpea roots harbor *Rhizobium* sp. that is able to fix nitrate, even in very poor soils Blade *et al.* [26]. Cowpea is attached by a number of pests, both on the field and in storage. If these pests are not controlled, they can cause up to 100% loss of crop yield Singh *et al.* [27]. Cowpea seeds in storage are significantly affected by the storage pest *C. maculatus* Adam and Baidoo [28]. This pest can damage about 50% of the seeds after only about four months in storage Caswell [29]. The readily available method of controlling cowpea pests is the application of chemical insecticide. The most important negative effects of the use of chemical insecticides are contamination of food and water sources, environmental health hazards to seed handlers and other nontargeted organisms. One alternative to the use of insecticides is planting of resistant cultivars which will limit the use of insecticides Ileke *et al.* [30, 31].

Baidoo and Mochiah [21] reported that some varieties of cowpea seeds that produced fewer *C. maculatus* adults recorded the largest weight reduction (20.91 % in just 42 days of storage). In contrast, Golob *et al.* [32] concluded that even though *C. maculatus* attacked cowpea, weight loss rarely exceeds 9% even after six months of storage.Research effort on the use of proper agronomic cultural practices as fertilization beside new cultivars with high yielding potentiality. Sowing date was one of the main agronomic practices that could directly affecton the level of insect infestation Abou-Zaid *et al.* [33].

Cowpea is also important because it fixes atmospheric nitrogen Asiwe *et al.* [34] and excess of its nitrogen requirements are made available for subsequent crop uptake thereby reducing the cost of nitrogen fertilization. Several management strategies are being employed in the suppression of these insect pests, including biological control Tamo *et al.* [35], chemical control Asiwe [36, 37], host plant resistance Fatokun *et al.* [38] and cultural method Asiwe [39] and Asiwe *et al.* [40]. The use of fertilizers as a yield booster has been reported Kutu *et al.* [41]. Also, some macro-nutrients, nitrogen, phosphorus and potassium have received some attention in the study of plant resistant to insect pests. Fertilizers not only improve crop yield, but also influence crop suitability for insect development, depending on the type of fertilizers and pest species Kogan [42]. In most of the rice growing areas in Asia, the greatest increases in population of major insect pests of rice were closely related to the long-term excessive application of nitrogen fertilizers Lu *et al.* [43].

El-Rodeny *et al.* [44] found appositive relationship between the physical-chemical characters of faba bean genotypes and infestation with *C. maculatus*. These characters must be taken in consideration for having resistant or tolerant seed of faba bean varieties to insect attack. Zein and Abo-Arab [45] reported that inoculation of bio-organic fertilizer and / or chemical fertilizer, nitrogen changed some physical and chemical characters of tested wheat varieties compared with control. Therefore the differences for susceptibility of insect infestation under the different level of nitrogen fertilizer may be due to this cause. The current findings are in harmony with those recorded earlier by Swella and Mushobozy [46] who conclude that the extent of weight loss may be due to damage of pulse beetle. The resistance of wheat is due to structure and composition of wheat such as, starches, carbohydrates and enzymes Evers *et al.* [47] and proteins Gupta *et al.* [48] to determine the levels of resistance of cultivars to grain insect infestation. They stated that the resistance of these cultivars might be attributed to the low content of protein and high content of carbohydrate compared to the susceptible cultivars. Ahmed *et al.* [49] reported that cultivars with hard seed coat showed non-preference by pulse beetle. El-Aidy *et al.* [50] indicated to significant differences between cultivars in dry matter, crude fiber, phenols, tannins and physical traits, thickness of hull and seed coat percentages. They reported that these parameters affected the degree of *C. maculatus* infestation.

The heavy application of nitrogen fertilizer rarely affect insect directly. However, it can alter or change morphological, biochemical and physiological characters of host plants and improve conditions for herbivores.

Nitrogen may influence semiochemicals and nutritional values of plants and also behavioral characteristics of herbivores Bentz *et al.* [51, 52]. It has been noted that a high rate of nitrogen fertilizer significantly increased the number of egg masses deposited by Asian corn borer, *Ostrinia furnacalis* on maize leaves Chu and Horng [53]. Nitrogen was found to modify the plant nutrition and reduce the resistance against aphid in cotton Cisneros and Godfrey [54]. Increasing the nitrogen availability in legumes may result in higher production of defense molecules, such as flavonoids, alkaloids, terpenes and sterols, which would in turn bolster the defense mechanisms against pests and result in beans with different levels of resistance against *C. maculatus* Potrich [55]. The decreased insect emergence may result from high larvae mortality caused by proteins with insecticidal potential, such as vicilin, which is present in some legumes Domingues *et al.* [56].

A loss of mass in stored beans is an important parameter to measure both from an economical point of view and as an indicator of cultivar resistance to pests Potrich [55]. This measure was positively correlated with the number of emerged insects, *i.e.* higher emergence resulted from higher consumption, or negatively correlated with the presence of substances capable of inhibiting the consumption of the seed interior by *C. maculatus* Costa and Boiça Júnior [57]. The infestation caused by *C. maculatus* was dependent on the cowpea cultivars and nitrogen sources Torres *et al.* [58]. The quantity, quality and proportion of nutrients present in the food (including nitrogen) and the presence of secondary or anti-nutritional compounds (allelochemicals) can have various impacts on the biology of insects, which affect their ability to contribute to the next generation and may have sublethal effects Parra and Panizzi [59]. Furthermore, the relationship between insect infestation and the chemical composition of seeds was investigated by many authors, Irshad *et al.* [60] and Warchalewski *et al.* [61].

Therefore, this paper reports the effects of applying four levels of nitrogen fertilizer (0.0, 16.5, 33.0 and 66.0 kg N fed^{-1}) in forms of ammonium nitrate(33 % N): 1- Before harvest (in field) on vegetative growth, yield, its components and seed chemical properties of cowpea variety Kafr El-Sheikh-1 at El-Balasy village, Sidi Salem Directorate, Kafr El-Sheikh Governorate, Egypt 2. Postharvest (in laboratory): the relationship between above mentioned parameters and the insect infestation by *Callosobruchus maculatus* through susceptibility and preferability tests.

MATERIALS AND METHODS

Two field experiments were conducted during two summer seasons of 2020 and 2021 at El-Balasy village, Sidi Salem Directorate, Kafr El-Sheikh Governorate, (31°33` North latitude, 30°78` East longitude and 3 m above the sea level) in the northern Delta of Egypt. The experiment was laid out in randomized complete block design with four replicates to study the effect of four nitrogen fertilizer rates (0.0, 16.5, 33.0 and 66.0 kg N fed^{-1})

Table 1: Chemical and physical characteristics of the experimental soil units after planting during 2020 and 2021 seasons

Chemical characteristics									
Season	pH (1:2.5)	EC (dSm^{-1})	ESP (%)	CEC (cmole kg^{-1})	OM %	$CaCO_3$ %	Available N $mgkg^{-1}$	Available P	Available K
2020	8.18	6.33	13.45	39.46	1.87	2.97	34	11.5	295
2021	8.25	6.80	13.90	39.50	1.75	2.61	38	10.2	310

Physical characteristics									
	Soil moisture characteristics					Particle size distribution			
Season	Field capacity (%)	Wilting point (%)	Available water (%)	Bulk density kg m^{-3}	Total porosity %	Sand (%)	Silt (%)	Clay (%)	Soil texture
2020	43.20	22.15	21.05	1.32	50.19	17.31	25.51	57.18	Clay
2021	42.80	21.25	21.55	1.28	51.70	16.95	26.13	56.92	Clay

on vegetative growth, yield, its component and seed chemical properties of cowpea variety Kafr El-Sheikh-1. Nitrogen fertilizer was applied in form of ammonium nitrate (33 % N) and divided into two equal parts and applied before the first and second irrigations in each season.

The preceding winter crop in two seasons was wheat (*Triticuma estivum*, L.). The plot area was 36 m^2 and contained ten ridges of 6 m long and 60 cm apart. Phosphorous fertilizer was applied in form of calcium super phosphate (12.5 % P_2O_5) at a rate of 150 kg fed^{-1} during soil preparation in each season. Kafr El Sheikh-1 cowpea seeds obtained from Hort. Dept., faculty of Agriculture, Kafr El Sheikh University. Cowpea seeds were inoculated by rhizobium bacteria just before planting. Cowpea planting was done by the local method of dibbling 4 seeds in each hill by hand with distance between hills was 20 cm apart at seeding rate of 20 kg seed fed^{-1} on May 23th and 20th of in the first season (2020) and the second season (2021), respectively. Cowpea plants were thinned on two plants $hill^{-1}$ after three weeks from sowing and harvested on 18th and 15th of September in the first and the second seasons, respectively. The other recommended agronomic practices of growing cowpea were applied in the manner prevailing in the region were practiced.

Soil texture of the experimental site was clay and salty of pH nearly of 8.2. Soil samples were taken before sowing of crop for chemical and mechanical properties analyses of the experimental soil were determined according to the standard procedures described by A.O.A.C. [62] and represented in Table 1.

Studied Parameters

Growth, Yield and its Components At harvest, ten plants were randomly chosen from the middle two ridges of each plot to determine plant height (cm), No. of branches $plant^{-1}$, No. of pods $plant^{-1}$, seed yield $plant^{-1}$ (g) and seed index (g). Then, ten pods from the previous ten plants were chosen randomly to estimate pod length (cm) and No. of seeds pod^{-1}. While, seed yield fed^{-1} (kg) were estimated from the whole cowpea plants in plot and adjusted at 12 % moisture content.

Seed Chemical Properties: Seeds samples were taken after harvest at random from each seeds of ten plants to determine some seed chemical properties:

- Seed nitrogen content (%) according to the modified micro Kjeldahl method was determined according to the methods of Association of Official Analytical Chemists described in A.O.A.C. [62].
- Seed crude protein content (%) was calculated by multiplying seed nitrogen content by 6.25 (Moore *et al.* [63].
- Seed phosphorus content (%), phosphorus was determined colorimetrically according to the methods described in A.O.A.C. [62].
- Seed potassium content (%), potassium was measured by flame photometer according to the methods described in A.O.A.C. [62].
- Seed total carbohydrates content (%). Total carbohydrates was determined in dry matter by using phenol-sulphuric acid method described by Dubios *et al*. [64] and calculated as % of dry weight.
- Seed crude fiber content (%). Crude fiber testing using the gravimetric method was done by Wali *et al*. [65].
- Seed fat content (%). Fat percentage was determined by using soxlet apparatus using petrolium ether as a solvent according to A.O.A.C. [66].
- Seed ash content (%), according to the methods described in A.O.A.C. [66] and Marshall [67].

Mass Culturing of *Callosobruchus maculatus*: Original stock of *C. maculatus* was obtained from Stored Product Pest Laboratory, Plant Protection Research Institute, Sakha Agricultural Research Station, Egypt. Subsequently culture of *C. maculatus* was reared on cowpea *Vigna unguiculata* L. Walp. Cowpea seeds were thoroughly cleaned to remove dirt and damaged seeds. They were sterilized by heating at 50°C for 6 hours to kill any prior infestation by insects. These seeds were then conditioned to a room temperature before being used for subsequent experiments. Approximately, 100-200 *C. maculatus* adults were reared in glass jar containing 300 g cowpea seeds. Jars were covered with muslin cloth, fastened with rubber bands, then placed in an incubator at 28±2°C, 70±5% relative humidity and light : dark photoperiod of 16: 8 h. Adult insects were allowed to feed and oviposit for 10 days then they were sieved out with a mesh size of 7 meshes. The cowpea seeds containing eggs were left until the new adult emerge. The subsequent F_1 progenies of the insects, which emerged from the culture were used for all experiments. All beetles used for all bioassays were of mixed sex and 0-2 days old.

Studied Parameters

Susceptibility of Cowpea Seeds (Non-choice Infestation): The susceptibility of cowpea seeds obtained from seeds prior treated in the field with nitrogen fertilizer levels (0.0, 16.5, 33.0 and 66.0 kg N fed^{-1}) were evaluated in the laboratory under non-choice conditions using 20 g of each treatment in glass jar (250 ml). Each jar was infested with ten pairs of newly emerged adults (0-2 days old) and the jar was covered with muslin cloth, four replicates were used for each treatment. The adults emerged were recorded and grain weight loss (%) was estimated according the following equation:

$$\text{Grain weight loss \%} = \frac{\text{Initial dry weight (g)-final dry weight (g)}}{\text{Initial dry weight (g)}} \times 100$$

Preferability Experiment (Free-Choice Infestation): In order to study preferability of cowpea seeds by *C. maculatus*, four separate choice chamber (four replicates) of each tested treatment were made using glass jars (70 x 70 x 20 cm in height) covered with a lid. Number of Petri-dishes (6 cm diameter) was according to the number of treatments, each containing 20 g of tested cowpea seeds was placed into prepared jars. Two hundred unsexed adults of newly emerged adults (0-2 days old) were released in each test chamber to give the insect a free-choice on any treatment. Number of insects harboured in each treatment after 5 days of releasing was used as an indicator for the preferability of tested cowpea seeds treatment for *C. maculatus*.

Statistical Analysis: The analysis of variance was carried out according to the procedure described by Gomez and Gomez [68]. Data were statistically analyzed according to using the MSTAT-C Statistical Software Package Freed [69]. Where the F-test showed significant differences among means Turkey's Honestly Significance Difference (H.S.D.) test at 0.05 level was used to compare between means.

RESULTS AND DISCUSSION

Growth, Yield and Yield Components: Results in Table 2, indicated that all vegetative growth, yield and yield components of cowpea were significantly increased with increasing nitrogen fertilizer rates from 0.0 to 16.5, 33.0 and 66.0 kg N fed^{-1} in both seasons. Cowpea plants treated with 66.0 kg N fed^{-1} significantly gave the maximum mean values of plant height (82.70 and 91.28 cm), No. of branches plant^{-1} (3.50 and 3.73), No. of pods plant^{-1} (26.35 and 29.48), pod length (17.63 and 18.10 cm), No. of seeds pod^{-1} (15.30 and 16.25), seed yield plant^{-1} (51.04 and 63.76 g), 100-seed weight (14.83 and 16.06 g) and seed yield fed^{-1} (819.00 and 885.75 kg) in both seasons, respectively. But, the differences between nitrogen fertilizer rates of 33.0 and 66.0 kg N fed^{-1} on No. of branches plant^{-1}, pod length (cm) and seed index (g) during both seasons as well as No. of pods plant^{-1}, No. of seeds pod^{-1} and seed yield fed^{-1} (kg) in the first season were not significant. The superiority ratios in the first season when cowpea plants treated with 16.5, 33.0 and 66.0 kg N fed^{-1} over without nitrogen added were 7.32, 13.15 and 23.57 % for plant height; 36.09, 50.00 and 52.17 % for No. of branches plant^{-1}; 41.34, 64.87 and 72.22 % for No. of pods plant^{-1}; 40.92, 55.63 and 62.07 % for pod length; 39.89, 65.45 and 71.91 % for No. of seeds pod^{-1}; 119.27, 219.02 and 254.85 % for seed yield plant^{-1}; 11.56, 20.19 and 24.68 % for 100-seed weight in addition to 63.17, 92.54 and 98.79 % for seed yield fed^{-1}, respectively. The excess ratios in the second season when cowpea plant received 16.5, 33.0 and 66.0 kg N fed^{-1} over without nitrogen application were 11.69, 16.72 and 25.90 % for plant height; 33.33, 45.45 and 50.51 % for No. of branches plant^{-1}; 80.86, 104.13 and 121.20 % for No. of pods plant^{-1}; 27.93, 46.91 and 54.37 % for pod length; 42.63, 56.84 and 71.05 % for No. of seeds pod^{-1}; 192.57, 288.36 and 341.73 % for seed yield plant^{-1}; 11.87, 19.39 and 24.42 % for 100-seed weight in addition to 43.40, 66.83 and 75.14 % for seed yield fed^{-1}, respectively. The increase in plant height associated with increasing nitrogen fertilizer rates may be attributed to the role of nitrogen in

Table 2: Mean values of growth, yield and yield components of cow pea as affected by nitrogen fertilizer rates during 2020 and 2021 seasons

Season	2020	2021	2020	2021	2020	2021	2020	2021
N rate (kg fed^{-1})	Plant height (cm)		No. of branches plant^{-1}		No. of pods plant^{-1}		Pod length (cm)	
0.0	66.93^{D}	72.50^{D}	2.30^{C}	2.48^{C}	15.30^{C}	13.33^{D}	10.88^{C}	11.73^{C}
16.5	71.83^{C}	80.98^{C}	3.13^{B}	3.30^{B}	21.63^{B}	24.10^{C}	15.33^{B}	15.00^{B}
33.0	75.73^{B}	84.63^{B}	3.45^{A}	3.60^{A}	25.23^{A}	27.20^{B}	16.93^{A}	17.23^{A}
66.0	82.70^{A}	91.28^{A}	3.50^{A}	3.73^{A}	26.35^{A}	29.48^{A}	17.63^{A}	18.10^{A}
H.S.D. at 5 %	3.05	3.17	0.30	0.27	1.80	1.61	1.23	1.04
N rate (kg fed^{-1})	No. of seeds pod^{-1}		Seed yield plant^{-1} (g)		100-seed weight (g)		Seed yield fed^{-1} (kg)	
0.0	8.90^{B}	9.50^{D}	14.38^{D}	14.43^{D}	11.89^{C}	12.91^{C}	412.00^{C}	505.75^{D}
16.5	12.45^{B}	13.55^{C}	31.54^{C}	42.23^{C}	13.27^{B}	14.44^{B}	672.25^{B}	725.25^{C}
33.0	14.73^{A}	14.90^{B}	45.89^{B}	56.06^{B}	14.29^{A}	15.41^{A}	793.25^{A}	843.75^{B}
66.0	15.30^{A}	16.25^{A}	51.04^{A}	63.76^{A}	14.83^{A}	16.06^{A}	819.00^{A}	885.75^{A}
H.S.D. at 5 %	1.10	1.26	4.74	5.26	0.79	0.69	66.5	40.4

Mean followed by the same letter are not significant according to H.S.D. Turkey's Honestly Significance Difference test at 5 % level

Table 3: Mean values of seed chemical properties as affected by nitrogen fertilizer rates

Season	2020	2021	2020	2021	2020	2021	2020	2021
N rate (kg fed^{-1})	N content (%)		Crude protein content (%)		P content (%)		K content (%)	
0.0	3.147^{D}	3.226^{D}	19.67^{D}	20.16^{D}	0.381^{C}	0.391^{C}	0.983^{D}	0.966^{C}
16.5	3.431^{C}	3.521^{C}	21.44^{C}	22.00^{C}	0.403^{B}	0.406^{B}	1.033^{C}	1.054^{B}
33.0	3.613^{B}	3.718^{B}	22.58^{B}	23.24^{B}	0.415^{AB}	0.423^{A}	1.075^{B}	1.107^{A}
66.0	3.720^{A}	3.824^{A}	23.25^{A}	23.90^{A}	0.424^{A}	0.428^{A}	1.102^{A}	1.122^{A}
H.S.D. at 5 %	0.082	0.102	0.51	0.64	0.014	0.011	0.026	0.031
N rate (kg fed^{-1})	Total carbohydrate content (%)		Crude fiber content (%)		Fat content (%)		Ash content (%)	
0.0	63.73^{A}	63.25^{A}	10.56^{A}	10.47^{A}	0.645^{C}	0.702^{D}	3.783^{C}	3.837^{C}
16.5	62.55^{B}	61.99^{B}	9.55^{B}	9.46^{B}	0.787^{B}	0.802^{C}	3.957^{B}	4.050^{B}
33.0	61.65^{C}	60.95^{C}	9.11^{C}	8.99^{C}	0.852^{A}	0.857^{B}	4.083^{A}	4.198^{A}
66.0	60.96^{D}	60.56^{C}	8.92^{C}	8.60^{D}	0.889^{A}	0.902^{A}	4.128^{A}	4.215^{A}
H.S.D. at 5 %	0.56	0.76	0.30	0.32	0.045	0.027	0.053	0.072

Mean followed by the same letter are not significant according to H.S.D. Turkey's Honestly Significance Difference test at 5 % level

enhancement meristematic activity and cell division, which caused increase in internodes length, No. of internodes and both of them. The increase in cowpea yield and its attributes because of increasing nitrogen fertilizer rates up to 33.0 or 66.0 kg N fed^{-1} can be easily ascribed to the role of nitrogen in activating plant growth, consequently enhancement yield components (No. of branches plant^{-1}, No. of pods plant^{-1}, pod length, No. of seeds pod^{-1}, seed yield plant^{-1} and seed index) and consequently increasing seed yield fed^{-1}. Many investigators came out with similar results as Abayomi *et al.* [7], Azarpour *et al.* [9], Moursi *et al.* [10], Singh *et al.* [11], Sebetha *et al.* [12], Mohammad and Mukhtar [13] and Shaban *et al.* [8].

Seed Chemical Properties: Results presented in Table 3, clearly show that the increase in nitrogen fertilizer rate from 0.0, 16.5 and 33.0 to 66.0 kg N fed^{-1} caused significantly increases in almost seed chemical properties of cowpea, except mean values of total carbohydrate content andcrude fiber content were significantly decreased by increasing nitrogen fertilizer rate during 2020 and 2021 seasons. Cowpea seeds which obtained from soil fertilized by 66.0 kg N fed^{-1} gave significantly the maximum mean values of nitrogen content (3.720 and 3.824 %), crude protein content (23.25 and 23.90 %), phosphorus content (0.424 and 0.428 %), potassium content (1.102 and 1.122 %), fat content (0.889 and 0.902 %) and ash content (4.128 and 4.215 %) in addition to recorded the lowest mean values of total carbohydrate content (60.96 and 60.56 %) andcrude fiber content (8.92 and 8.60 %) in both seasons, respectively but there is no significant difference among application of 33.0 kg N fed^{-1} and 66.0 kg N fed^{-1} in almost seed chemical properties under study. The superiority ratios in the first season when cowpea plants treated with 16.5, 33.0 and 66.0 kg N fed^{-1} over without nitrogen added were 9.04, 14.81 and 18.23 % for crude protein content in addition to 4.61, 7.94 and 9.12 % for ash content, respectively. The excess ratios in the second season when cowpea plant received 16.5, 33.0 and 66.0 kg N fed^{-1} over without nitrogen application were 9.15, 15.28 and 18.55 % for crude protein content in addition to 5.56, 9.42 and 9.85 % for ash content, respectively. The increases in seed nitrogen content or seed crude protein content by raising nitrogen rates may be due to the fact that nitrogen is essential for building up to the protoplasm amino acids and proteins. These results are in agreement with that obtained by Adeyemi *et al.* [4], Moursi *et al.* [10], Mukhtar *et al.* [14], Amujoyegbe and Alofe [15], Musa *et al.* [16], Daramy *et al.* [17], El-Atawy and Kasem [18] and Muneta *et al.* [19].

Infestationof Cowpea Seeds by *Callosobruchus maculatus*: Two bioassay experiments were conducted to evaluate the effect of the different nitrogen fertilizer levels on the response of *C. maculatus* beetles postharvest. The first was non-choice assay to determine the parameters of susceptibility (egg oviposition, hatchability% and number of adult emergence) while the second was to identification the preferred nitrogen rate for *C. maculatus* (choice test). In addition, weight loss and the net weight loss % were evaluated at different rates of nitrogen fertilizer. Results in Table 4, showed that all criteria belong to susceptibility were significantly influenced by the nitrogen levels. All the tested parameters increased by increasing of nitrogen from 0.0 to 66.0kg fed^{-1}. For example, mean number of adult emergence increased from 27.0 to 42.3 adult at the levels of 16.5 and 66.0 kg N fed^{-1}. The same trend was obtained with the preferability where the cowpea seeds obtained from soil previously received the highest rate of nitrogen (66.0 kg fed^{-1}) harboured the highest number of cowpea beetles compared to the other levels of nitrogen. Also, results showed that there were significant differences between the treatment in respect to the number of adult emergence and the harboured adults.

Results summarized in Table 5, indicated the significant effect of nitrogen levels on both seed weight loss % and net seed yield (kg fed^{-1}). 66.0 kg N fed^{-1} level achieved the highest yield (852.4 kg fed^{-1} as mean of two seasons) and the highest percent of weight loss (19.55%) following by the level of 33.0 kg N fed^{-1} with 818.5kg fed^{-1} and 15.5% loss. Based on the obtained net yield (kg fed^{-1}), the level 33.0 kg N fed^{-1} is considered the best where it achieved the highest net yield. This variation between the weight loss % may be attribute to the chemical composition of seeds obtained of this treatment and the number of emerged adults.

This results perhaps elucidates the effect of chemical composition arises from the nitrogen level on the level of insect infestation with cowpea beetles.

Results in Table 6, indicated that the all tested parameters of susceptibility (egg oviposition, egg hatchability %, number of adult emergence (F_1) and seed weight loss %) and preferability (No. of insects in each treatment) beside net seed yield (kg fed^{-1}) were highly affected by the levels of nitrogen fertilization. In addition there were significant differences between the tested parameters at the different level of N fertilizer.

Data in Table 7, also indicated that the all tested parameters of choice and non-choice had highly significant positive correlation with the chemical components of cowpea seeds except total carbohydrate content (%) and crude fiber content (%), which showed negative correlation with the aspects of cowpea beetles (F_1 and net weight loss). Eventually, the current study showed the net yield postharvest and the effect of nitrogen fertilization on the degree of infestation by cowpea beetles. The present study revealed that the best considerable level of nitrogen was of 33.0 kg N fed^{-1} fertilizer although the level of 66.0 kg N fed^{-1}achieved the highest harvest of seed yield.

The obtained findings demonstrated that the highest doses of N fertilizer caused high weight loss (%) causing high reduction of cowpea seeds net yield.

Results in the current study are agreement with that of Andrew *et al.* [70], Bi *et al.* [71] and Biswas *et al.* [72] they reported that nitrogen plays an important role on the intensity of insect pests. They found a direct correlation of yield and nitrogen. Many studies suggest that fertilizer may affect the physiologically susceptibility of a crop to pest Magdoff [73]. Song *et al.* [74] found that nitrogen is essential to crop yield improvement and it can change crops ability to defend against herbivores. Plant nitrogen and secondary defensive chemicals are known to affect the performance of phytophagous insects, because the quality of host plant consumed by insect pests often affect their intake, growth rate, development time, survivorship and fecundity etc. Chen *et al.* [75] and Wu *et al.* [76]. Additionally high nitrogen fertilization changed the resistance of poplar to *Lymantria dispar* after continuous feeding test Glynn *et al.* [77].

The information gained from the study could be important for avoiding the miss or over use of different fertilizers which would lead to produce more susceptible cowpea seeds to infestation with the test insect, *C. maculatus.* It is also possible not use or reduce the use of pesticides when using certain levels of nitrogen fertilization. So, as a step in this line of research, the goals of the present work were: to study the effect of different levels of nitrogen applied to Kafr El-Sheihk cowpea variety in the field on the degree of infestation with

Table 4: Effect of nitrogen fertilizer rates for susceptibility (Non-choice) and preferability (choice) to infestation by *Callosobruchs maculatus*, Fab on cowpea seeds in the 2020 and 2021 seasons

	Non – choice (Susceptibility)						Choice (Preferability)					
	Egg oviposition			Eggs hatchability (%) **			No. of adults emergence (F1)			No. of insects after 5 days release		
N kg fed^{-1}	2020	2021	Mean	2020	2021	Mean	2020	2021	Mean	2020	2021	Mean
0.0	100.0^D	107.0^D	103.5	70.00^C	71.10^C	70.55	14.00^D	17.00^D	15.50	18.00^D	20.67^D	19.33
16.5	125.0^C	138.0^C	131.5	81.00^B	82.30^B	81.65	25.00^C	29.00^C	27.00	21.33^C	23.33^C	22.33
33.0	205.0^B	261.0^B	233.0	90.50^A	92.40^A	91.45	33.00^B	41.00^B	37.00	30.33^B	33.33^B	31.83
66.0	307.0^A	343.0^A	325.0	93.70^A	95.20^A	94.45	39.00^A	45.60^A	42.30	39.67^A	40.00^A	39.84
H.S.D. at 5 %	9.62	9.72		5.62	3.47		3.14	4.07		1.90	1.70	

Mean followed by the same letter are not significant according to H.S.D. Turkey's Honestly Significance Difference test at 5 % level

$$** \ \% \text{ Egg hatchability} = \frac{\text{No. of Egg oviposition}}{\text{Total No. of egg oviposition}} \times 100$$

Table 5: Effect of nitrogen fertilizer rates on seed yield, seed weight loss % and net seed yield of cowpea during 2020 and 2021 seasons

Trait	Seed yield (kg fed^{-1})			Seed weight loss %			Net seed yield (kg fed^{-1})		
N kg fed^{-1}	2020	2021	Mean	2020	2021	Mean	2020	2021	Mean
0.0	412.00^C	505.75^D	458.88	9.50^D	8.50^D	9.00	372.86^C	462.76^C	417.81
16.5	672.25^B	725.25^C	698.75	14.00^C	12.50^C	13.25	578.19^B	634.57^B	606.38
33.0	793.25^A	843.75^B	818.50	16.50^B	14.50^B	15.50	662.37^A	721.31^A	691.84
66.0	819.00^A	885.75^A	852.38	20.10^A	19.00^A	19.55	654.40^A	717.46^A	685.93
H.S.D. at 5 %	66.46	40.40		0.96	1.93		60.02	28.03	

Mean followed by the same letter are not significant according to H.S.D. Turkey's Honestly Significance Difference test at 5 % level

Table 6: Analysis of variance (mean square) for insect traits on cowpea seeds as affected by nitrogen fertilizer during 2020 and 2021 seasons

		2020 season			2021 season		
Source of variance		Replication	Level of nitrogen	Error	Replication	Level of nitrogen	Error
Degree of freedom		3	3	9	3	3	9
Non-choice	Egg oviposition	18.750	34696.917**	18.972	55.167	48083.667**	19.389
	Eggs hatchability (%)	8.042	454.907**	6.486	18.847**	478.733**	2.473
	No. of adults emergence (F1)	3.583	467.667**	2.028	2.282	659.560**	3.393
Choice	No. of insects after 5 days release	0.846	120.993**	0.744	1.006	152.193**	0.591
Seed weight loss %		1.635**	32.010**	0.191	0.390	39.583**	0.768
Seed yield (kg fed^{-1})		3105.417	138523.417**	906.361	976.417	116130.250**	334.861
Net seed yield (kg fed^{-1})		2032.988	95763.211**	739.152	875.578**	84214.951**	161.187

Table 7: Simple correlation coefficients between seed chemical properties and insect traits from the average of all data during 2020 and 2021 seasons

	N content (%)	Crude protein content (%)	P content (%)	K content (%)	Total carbohydrate content (%)	Crude fiber content (%)	Fat content (%)	Ash content (%)
Egg oviposition	0.903^{**}	0.903^{**}	0.854^{**}	0.886^{**}	-0.905^{**}	-0.857^{**}	0.872^{**}	0.874^{**}
Eggs hatchability (%)	0.945^{**}	0.944^{**}	0.913^{**}	0.941^{**}	-0.927^{**}	-0.932^{**}	0.957^{**}	0.932^{**}
No. of adults emergence (F1)	0.974^{**}	0.973^{**}	0.925^{**}	0.944^{**}	-0.958^{**}	-0.947^{**}	0.941^{**}	0.969^{**}
No. of insects after 5 days release	0.398^{*}	0.398^{*}	0.436^{*}	0.418^{*}	-0.389^{*}	-0.387^{*}	0.390^{*}	0.479^{**}

cowpea beetle, *C. maculatus* in laboratory. The work also, involved the study of change in the chemical composition and its relation to the degree of infestation with the mentioned insect.

CONCLUSION

This study showed a significant effect of the application of nitrogen fertilizer on the growth, seed yield and seed chemical properties of cowpea variety Kafr El-Sheikh-1. The highest seed yield fed^{-1} and quality were obtained from cowpea plants treated with 66.0 kg N fed^{-1} in both seasons without significant differences with 33.0 kg N fed^{-1} on almost cowpea traits under study. It could be concluded that cowpea treated with 66.0 or 33.0 kg N fed^{-1} in order to improve cowpea yield and seed quality under the condition of El-Balasy village, Sidi Salem Directorate, Kafr El–Sheikh Governorate, Egypt. This investigation clarified that the N fertilizer can increase the resistance of cowpea seeds and reduces the insect infestation by cowpea beetle through the effect on the chemical components of seeds. Also, this study limited the suitable level of nitrogen fertilizer necessary to obtain the highest yield with the least insect infestation. In addition those breading research workers can utilize these results for reducing the overuse of N fertilizers.

REFERENCES

1. Agbogidi, O.M., 2010. Response of six cultivars of cowpea [*Vigna unguiculata* (L.) Walp.] to spent engine oil. African Journal of Food Science and Technology, 1(6): 139-142.
2. El Naim, A.M. and A.A. Jabereldar, 2010. Effect of plant density and cultivar on growth and yield of cowpea [*Vigna unguiculata* (L.) Walps.]. Australian J. Basic and Appl. Sci., 4(8): 3148-3153.
3. El Naim, A.M., A.A. Jabereldar, S.E. Ahmed, F.M. Ismaeil and E.A. Ibrahim, 2012. Determination of suitable variety and plants per stand of cowpea [*Vigna unguiculata* (L.) Walps.] in the sandy soil, Sudan. Advances in Life Sci., 2(1): 1-5.
4. Adeyemi, S.A., F.B. Lewu, P.O. Adebola, G. Bradley and A.I. Okoh, 2012. Protein content variation in cowpea genotypes [*Vigna unguiculata* (L.) Walp.] grown in the in the Eastern Cape province of south Africa as affected by mineralized goat manure. African J. Agric. Res., 7(35): 4943-4947.
5. Ngalamu, T., J. Odraand N. Tongun, 2014. Cowpea production handbook. 1st Edition, Afristar Publishing House Juba, College of Natural Resources and Environmental Studies, University of Juba.
6. Madukwe, D.K., I.E. Christo and M.O. Onuh, 2008. Effects of organic manure and cowpea [*Vigna unguiculata* (L.) Walps.] varieties on the chemical properties of the soil and root nodulation. Sci. world J., 3 (1): 43-46.
7. Abayomi, Y.A., T.V. Ajibade, O.F. Sammuel and B.F. Sa'adudeen, 2008. Growth and yield responses of cowpea [*Vigna unguiculata* (L.) Walp] genotypes to nitrogen fertilizer application in the Southern Guinea Savanna Zone of Nigeria. Asian J. Plant Sci., 7(2): 170-176.
8. Shaban, A.M.I., A.E.M. Shokr, E.M. El-Tantawy and A.I. El-Kassas, 2019. Effect of nitrogen and phosphorus rates on cowpea growth and yield under El-Arish region conditions. 9th International Conference for Sustainable Agricultural Development 4-6 March 2019, Fayoum J. Agric. Res, & Dev., 33(1B): 133-145.
9. Azarpour, E., R.K. Danesh, S. Mohammadi, H.R. Bozorgi and M. Moraditochaee, 2011. Effects of nitrogen fertilizer under foliage spraying of humic acid on yield and yield components of cowpea [*Vigna unguiculata* (L.) Walps.]. World Appl. Sci. J., 13: 1445-1449.
10. Moursi, E.A., M.A. Aziz, M.A. Aiad and R.Kh. Darwesh, 2013. Effect of water stress, biofertilizers and nitrogen application rates on cowpea yield and some water relations in the North Middle Nile Delta region. J. Soil Sci. and Agric. Eng., Mansoura Univ., 4(11): 1289-1311.
11. Singh, A.K., P.N. Tripathi and R. Singh, 2007. Effect of Rhizobium inoculation, nitrogen and phosphorus levels on growth, yield and quality of kharif cowpea [*Vigna unguiculata* (L.) Walps.]. Crop Res., 33(1, 2 & 3): 71-73.
12. Sebetha, E.T., A.T. Modi and L.G. Owoeye, 2015. Cowpea crude protein as affected by cropping system, site and nitrogen fertilization. J. Agric. Sci., 7(1): 224-234.
13. Mohammad, M. and F.B. Mukhtar, 2018. Effect of nitrogen fertilizer treatments on duration of senescence, harvest time and yield in some varieties of cowpea [*Vigna unguiculata* (L.) Walps.]. Afr. J. Plant Sci., 12(3): 37-46.
14. Mukhtar, F.B., M. Mohammed and H.A. Ajiegbe, 2010. Effect of farmyard manure on senescence, nitrogen and protein levels in leaves and grains of some cowpea varieties. Bayero J. Pure and Appl. Sci., 3(1): 96-99.
15. Amujoyegbe, B.J. and C.O. Alofe, 2003. Influence of poultry manure and inorganic nitrogen fertilizer on grain yield and proximate component of two cultivars of cowpea [*Vigna unguiculata* (L.) Walps.]. Moor J. Agric. Res., 4(1): 37-45.
16. Musa, E.M., E.A.E. Elsheikh, I.A.M. Ahmed and E.E. Babiker, 2011. Effect of intercropping, *Brady rhizobium* inoculation and N, P fertilizers on yields, physical and chemical quality of cowpea seeds. Front. Agric. China, 5(4): 543-557.
17. Daramy, M.A., J. Sarkodie-Addo and G. Dumbuya, 2017. Effect of nitrogen and phosphorus fertilizer application on growth and yield performance of cowpea in Ghana. J. Exper. Biol. and Agric. Sci., 5(1): 38-44.
18. El-Atawy, Gh.Sh. and M.H. Kasem, 2011. Effect of deficit irrigation and nitrogen fertilization on cowpea yield, it components and water productivity in North Delta of Egypt. J. Soil Sci. and Agric. Eng., Mansoura Univ., 2 (3): 279-293.

19. Muneta, M.G., F. Mtambanengwe, M.J. Watts, M.R. Broadley, L.R. Murray and P. Mapfumo, 2020. Nitrogen effect on zinc biofortification of maize and cowpea in Zimbabwean smallholder farms. Agron. J., 112: 2256-2274.
20. Conway, G.R., 1997. The doubly green revolution: Food for all in the 21st Century. New York: Cornell University Press.
21. Baidoo, P.K. and M.B. Mochiah, 2014. Varietal susceptibility of improved cowpea *Vigna unguiculata* (L.) (Walp.) cultivars to field and storage pests. Sustainable Agric. Res., 3(2): 69-76.
22. Kabululu, M.S., 2008. Cowpea, (*Vigna unguiculata*) variety mixtures for stable and optimal leaf and seed yield when intercropped with maize in Central Tanzania. M.Sc. Thesis, Georg-August-Unuversitat, Gottingen, Germany, pp: 75.
23. Uzogara, S.G. and Z.M. Ofunya, 1992. Processing and utilization of cowpea in developing countries: A review. J. Food Proc. & Pres., 16(2): 105-147.
24. Bressani, R., 1985. Nutritive value of cowpea: In cowpea Research, Production and Utilization. In S. R. Singh & K. O. Rachie (Eds.) (pp: 353-359). John Wiley and Sons Ltd.
25. Timko, M.P., J.D. Ehlers and P.A. Roberts, 2007. Cowpea. In C. Kole (Ed.) Genome mapping and molecular breeding in plants, pulses, sugar and tuber Crops, 3: 49-65.
26. Blade, S.F., S.V.R. Shetty, T. Terao and B.B. Singh, 1997. Recent developments in cowpea cropping systems research. In B. B. Singh & M. Raj (Eds.), Advances in Cowpea Research. Exper. Agric., 34: 475-486.
27. Singh, S.R., L.E.N. Jackai, J.H.R. Dos Santos and C.B. Adalla, 1990. Insect pests of cowpea. In S. R. Singh (Ed.) Insect Pests of tropical Food Legumes (pp: 43-89). London: John Wiley and Sons Chichester.
28. Adam, J.I. and P.K. Baidoo, 2008. Susceptibility of five cowpea (*Vigna unguiculata*) varieties to attack by *Callosobruchus maculatus* (Fab.) [Coleoptera: Bruchidae]. J. Ghana Sci. Asso., 10(2): 85-92.
29. Caswell, G.H., 1984. The value of the pod in protecting cowpea seed from attack by bruchid beetles. Samaru J. Agric. Res., 2: 49-55.
30. Ileke, K.D., J.M. Adesina and E.O. Obajulaye, 2016. Synergetic effects of two botanicals entomocides as pest-protectants in maize grains. J. Biolo. Res., 89: 33-39.
31. Ileke, K.D., 2019. Insecticidal toxicity of two bruchid-resistant cowpea cultivar powders as cowpea seed protectants against *Callosobruchus maculatus* (Fab.) (Coleoptera: Chrysomelidae). Food Qual. & Saf., 3(1): 35-39.
32. Golob, P., R. Strinfellow and E.O. Asante, 1996. A review of the storage and marketing systems of the major food grains in Northern Ghana, NRI, 11: 64.
33. Abou-Zaid, G.G., S.M. Mostafa and R.A. El-Refaey, 2017. Genotype X Environment interaction effect on heritability and genetic advance for yield and its components of some faba bean genotypes. J. Plant Production, Mansoura Univ., 8(6): 665-669.
34. Asiwe, J.A.N., A. Belane and F.D. Dakora, 2009. Evaluation of cowpea breeding lines for nitrogen fixatrion at ARC-Grain Crops Institute, Potchefstroom, South Africa. Paper presented at the 16th International Congress on Biological Nitrogen Fixation, Montana, USA, 14-19 June, 2009.
35. Tamo, M., H. Bottenberg, D. Arodokoun and R. Adeoti, 1997. The feasibility of classical biological control of two major cowpea insect pests. In: Singh, BB, Mohan Raj Dashiell, KE Jackai, LEN (eds) Advances in cowpea research. Sayce Publishing, Devon, UK, pp: 259-270.
36. Asiwe, J.A.N., 2009. Insect mediated out crossing and gene flow in cowpea *Vigna unguiculata* (L.) Walp): implication for seed production and provision of containment structures for GT Cowpea. Afr. J. Biotechnol., 8(2): 226-230.
37. Asiwe, J.A.N., 2009. The impact of phosphate fertilizer as a pest management tactic in four cowpea varieties. Afr. J. Biotechnol., 8(24): 7182-7186.
38. Fatokun, C.A., P. Perrino and N.Q. Ng, 1997. Wide crossing in African *Vigna*species. In: Singh, BB, Mohan Raj Dashiell, KE Jackai, LEN (eds). Advances in Cowpea Research. Sayce Publishing, Devon, UK. pp: 50-57.
39. Asiwe, J.A.N., 2004. Impact of cultural practices in the management of major insect pests of cowpea (*Vigna unguiculata* (L.) Walp) Ph.D. Thesis, Univ. Ibadan, Ibadan, Nigeria, pp: 188.
40. Asiwe, J.A.N., S. Nokoe, L.E.N. Jackai and F.K. Ewete, 2005. Does varying cowpea spacing provide better protection against cowpea insect pests? Crop Prot., 24(5): 465-471.

41. Kutu, F.R., W. Deale and J.A.N. Asiwe, 2009. Assessment of maize and dry bean productivity under different intercropping systems and fertilizer regimes. Paper Accepted for presentation at 9th International Conference of African Crop Science Society, Cape.
42. Kogan, M., 1994. Plant resistance in pest management. In: Metcalf, R and Luckmann (eds.), Introduction to pest management, John Wiley and Sons, Inc. New York, pp. 73-128.
43. Lu, Z.X., X.P. Yu, K.L. Heong and H.U. Cui, 2007. Effect of nitrogen fertilizer on herbivores and its stimulation to major insect pests in rice. Rice Sci., 14(1): 56-66.
44. El-Rodeny, W.M., A.A. Salem, S.M. Mostafa and A.M. Mohamed, 2018. Comparative resistance as function of physical and chemical properties of selected faba bean promising lines against *Callosobruchus maculatus* postharvest. J. Plant Production, Mansoura Univ., 9(7): 609-617.
45. Zein, F.I. and R.B. Abo-Arab, 2000. Combined effect of bio-organo-fertilization at different N-levels on: 2-the degree of insect infestation by *Sitophilus oryzae* L. and *Rhizopertadominica* F. J. Agric. Sci. Mansoura Univ., 25(7): 4637-4645.
46. Swella, B.G. and D.M.K. Mushobozy, 2007. Evaluation of the efficacy of protestants against cowpea bruchids (*Callosobruchus maculatus* (F.) on cowpea seeds (*Vigna unguiculata* (L.) Walp.). Plant Protection Sci., 43: 68-72.
47. Evers, A.D., A.B. Blakeney and L. Obrien, 1999. Cereal structure and composition. Aust. J. Agric. Res., 50: 629-650.
48. Gupta, A.K., S.R. Bahal, B.K. Awasthi and R.A. Verma, 2000. Reaction of protein, starch and ash constituent of different varieties of maize on growth and development of *Sitophilus oryzae* (L.). Indian J. Entomology, 62: 375-381.
49. Ahmed, K., F. Khalique, I.A. Khan, M. Afzal and B.A. Malik, 1993. Genetic differences for susceptibility of chickpea tobruchid beetle (*Callosobruchus chinensis* L.) attack. Pak .J. Sci. Ind. Res., 36: 96-98.
50. El-Aidy, N.A., H.M. El-Zun, E.A.I. Mohamed and A.A. Ashrei, 2008. Susceptibility of some faba bean cultivars to cowpea weevil infestation as affected by chemical, physical and viability traits of seeds. Proc. 2nd Field Crops Conf., FORI, ARC, Giza, Egypt, 14-16 Oct., 407-417.
51. Bentz, J.A., I.I.I.J. Reeves, P. Barbosa and B. Francis, 1995.Within-plant variation in nitrogen and sugar content of poinsettia and its effects on the oviposition pattern, survival and development of *Bemisia argentifolii* (Homoptera: Aleyrodidae). Environ Entomol., 24: 271-277.
52. Bentz, J.A., I.I.I.J. Reeves, P. Barbosa and B. Francis, 1995. Nitrogen fertilizer effect on selection, acceptance and suitability of *Euphorbia pulcherrima* as a host plant to Bemisiatabaci. Environ. Entomol., 24: 40-45.
53. Chu, Y.I. and S.B. Horng, 1994. Effect of slag and nitrogen fertilizer on the damage of Asian corn borer to field corn. Mem. Coll. Agric., Natl. Taiwan Univ., 34(1): 45-53.
54. Cisneros, J.J. and L.D. Godfrey, 2001. Midseason pest status of the cotton aphid in California cotton: Is nitrogen a key factor? Environ. Entomol., 30: 501-510.
55. Potrich, M., 2006. Associação de variedadesresistentes de milho e fungosentomopatogênicos para o controle de Sitophilus spp [Association of resistant corn varieties and entomopathogenic fungi for the control of Sitophilus ssp.]. MSc thesis. UniversidadeEstadual do Oeste do Paraná, MarechalCândido Rodon Campus, Paraná (2006).
56. Domingues, S.J.S., F.R. Melo, J.M. Aguiar, A.G. Affonso, J.S. Giuli, J.L. Rose, M.P. Sales, L.F. Machado, C.R. Azevedo, P.C. da Cunha and A.F. Uchoa, 2006. Resistance of *Vigna unguiculata* (cowpea) seeds to *Callosobruchus maculatus* is restricted to cotyledonary tissues. J. Sci. Food Agric., 86(12): 1977-1985.
57. Costa, N.P.D. and A.L. BoiçaJúnior, 2004.Efeito de genótipos de caupi, *Vigna unguiculata* (L.) Walp., sobre o desenvolvimento de *Callosobruchus maculatus* (Fabricius) (Coleoptera: Bruchidae). Neotrop. Entomol., 33: 77-83.
58. Torres, E.B., R.S.A. Nóbrega, P.I. Fernandes-Júnior, L.B. Silva, G.D.S. Carvalho, R.D.C.N. Marinho and B.E. Pavan, 2016. The damage caused by *Callosobruchus maculatus* on cowpea grains is dependent on the plant genotype. J. Sci. Food Agric., 96 (12): 4276-4280.
59. Parra, J.R.P. and A.R.A.Panizzi, 2009.Bioecologia e nutrição de insetos: base para o manejointegrado de pragas [Bioecology and insect nutrition as basis for integrated pest management]. Brasília, DF: Embrapa Informação Tecnológica, Londrina. Embrapa Soja-Capítuloemlivrocientífico (ALICE):1107-1139.

60. Irshad, M., W.A. Gillani and A. Khan, 1988. Maize grain resistance to *Sitotroga cerealella* and *Sitophilus oryzae*. Pakistan J. Agric. Res., 9: 539-542.
61. Warchalewski, R., D. Piasecka-kwiatkowska, J. Nawrot and Z. Winiecki, 1993. Natural protection system of cereal rain against storage pests-myth or fact? Ochrona-Rodlin., 37(10): 11-12.
62. A.O.A.C., 2005. Official Methods of Analysis of the Association of Official Analytical Chemists. Published by A.O.A.C. 16th Ed., Washington, D.C., U.S.A.
63. Moore, J.C., J.W. DeVries, M. Lipp, J.C. Griffiths and D.R. Abernethy, 2010. Total protein methods and their potential utility to reduce the risk of food protein adulteration. Compr. Rev. Food Sci. Food Saf., 9(4): 330-357.
64. Dubios, M., K.A. Gilles, J.K. Hamilton, P.A. Rebens and F. Smith, 1956. Colorimetric method for determination sugars and related substances. Anal. Chem. Soc., 46: 1662-1669.
65. Wali, M., N.F. Haneda and N.Maryana, 2014. Identifikasi Kandungan Kimia Bermanfaat pada Daun Jabon Merah dan Putih (*Anthocephalus spp*.) Identifcation of useful chemical content of red and white jabon leaf (*Anthocephalus spp*.). J. Silvikultur Tropika, 5(2): 77-83.
66. A.O.A.C., 1990. Official Method of Analysis, 15th Ed., Association of Official Analytical Chemists, Inc., USA.
67. Marshall, M.R., 2010. Ash Analysis. Food Analysis. Boston, MA, Springer US: 105-115.
68. Gomez, K.A. and A.A. Gomez, 1984.Statistical procedures for agricultural research. 2nd, (ed). John Wiley and Sons, NY, U.S.A.
69. Freed, R.D., 1991. MSTATC Microcomputer Statistical Program. Michigan State University, East Lansing, Michigan, USA.
70. Andrew, G.L., G. Cooke, R.D. Meeks, P. Dugger and D. Richter, 2000. The interaction of nitrogen fertilization and insect populations. Proc. Beltwide Cotton Conf., USA., 2(1): 993-996.
71. Bi, J.L., N.C. Toscano and M.A. Madore, 2003. Effect of urea fertilizer application on soluble protein and free amino acid content of cotton petioles in relation to the silver leaf whitefly (*Bemisia argentifolii*) population. J. Chem. Ecol., 29(3): 747-761.
72. Biswas, S., B. Mahato, P. Panda and S. Guha, 2009. Effect of different doses of nitrogen on insect pest attack and yield potentiality of Okra, *Abelmonschus esculentus* (L.) Moench at terai ecology of West Bengal. J. Ent. Res., 33(3): 219-222.
73. Magdoff, R.R., 1992. Building soil for better crops. Organic Matter Management. University Nebraska Press, Lincoln, USA, ppp: 176.
74. Song, Y., J. Liu and F. Chen, 2020. *Azotobacter chroococcum* inoculation can improve plant growth and resistance of maize to armyworm, *Mythimna separata* even under reduced nitrogen fertilizer application. Pest Manag. Sci., 76(12): 4131-4140.
75. Chen, F.J., G. Wu, F. Ge, M.N. Parajulee and R.B. Shrestha, 2005. Effects of elevated CO_2 and transgenic Bt cotton on plant chemistry, performance and feeding of an insect herbivore, the cotton bollworm. Entomol. Exp. Appl., 115: 341-350.
76. Wu, G., F. Chen, Y. Sun and F. Ge, 2007. Response of successive three generations of cotton bollworm, *helicoverpa armigera* (hübner), fed on cotton bolls under elevated CO_2. J. Environ. Sci., 19: 1318-1325.
77. Glynn, C., D.A. Herms and M. Egawa, 2003. Effects of nutrient availability on biomass allocation as well as constitutive and rapid induced herbivore resistance in poplar. Oikos, 101: 385-397.