

Full Length Research Paper

Effects of biopesticides on developmental stages and longevity of *Callosobruchus maculatus* in some leguminous grains

Ododego V. Felicia

¹Department of Zoology and Environmental Biology, Faculty of Biological Sciences, University of Nigeria, Nsukka, Enugu State, Nigeria.

²Department of Plant Science and Biotechnology, Faculty of Biological Sciences, University of Nigeria, Nsukka, Enugu State, Nigeria.

Accepted 10, February 2014

The study deals with the effects of biopesticides or botanical treatments and a synthetic chemical (pirimiphos methyl) on the developmental stages and longevity of *Callosobruchus maculatus* in *Vegan unguiculata*, *V. subterranea* and *Cajanus cajan* and to suppress or delay the development of this pest. The biopesticide concentrations were 0, 0.2, 0.4, 0.6, 0.8 and 1.0 g per 20 g of each grain and that of synthetic chemical was 0.1 g per 20 g of each grain. The result revealed that the highest delay in development of *C. maculatus* was recorded with pirimiphos methyl, also development, was observed only at egg stage where it took 40 days for the *C. maculatus* egg to emerge on *V. unguiculata*, 38 days for its emergence on *C. cajan* and 37 days for the egg to emerge on *V. subterranea* while all other stages of development viz larva, pupa and adult could not emerge with pirimiphos methyl. The botanicals used were all effective when compared with the control. In *C. cajan*, *A. sativum* caused highest delay in *C. maculatus* development viz egg 15.00 days, larva 22.00 days, pupa 33.00 days and adult 38.00 days. Also, in *V. subterranea*, *A. sativum* delayed development of *C. maculatus* at the highest dose rate viz egg 13.00 days, larva 19.00 days, pupa 33.00 days and adult 37.00 days. *Capsicum nigrum* treatment was the 2nd best in delaying or reducing development of *C. maculatus* at the highest dosage rate in all the grains. All other treatments behaved alike. The efficacy and performance of these treatments also showed that *A. sativum* and *C. nigrum* delayed development of *C. maculatus* more than all other treatments. From the findings of this study it is evident that *A. sativum* and *C. nigrum* are very effective in delaying *C. maculatus* development and longevity and therefore could be recommended in development and longevity studies.

Key words: Biopesticides, longevity, developmental stages, *Callosobruchus maculatus*, legumes, pirimiphos methyl.

INTRODUCTION

The polyphagous pest *Callosobruchus maculatus* is the single most damaging insect pest of leguminous grains in

most tropics and subtropics. Its larvae infested grains such as beans, chick pea, green gram, lentil, broad bean and green pea. Females lay single eggs on the surface of grains sheath in field or on dried seeds in stores of grain legumes. The hatched larva bores the seed below the lower surface of eggs and the egg shell remains glued to the bean. The larval developmental period is different by physical conditions (Bagheri-Zenouz, 1996; Ekeh et al., 2014; Singh, 2011). Larvae remain inside the seed and the appearance of a capped exit hole on the seed shows the pupal stage (Right-Assia et al., 2010).

The adults do not need food or water and can reproduce immediately after emergence (Ekeh et al., 2014; Majeed, et al., 2006). *C. maculatus* causes serious damage to grains such that about 30% of the total grains are lost to it annually. Since legumes provide the cheapest and the richest source of plant protein and are supposed to be poor man's meal, control of this pest is essential (Singh, 2011). The heavy infestation of grains by these insects makes them lose their germination capacity and therefore become unfit for human consumption. Hence in order to reduce the infestation of this pest on these grains our research was carried out to test the effectiveness of medicinal plants as an alternative to chemical control with its attendant problems such as health hazard, insect resistance, pest resurgence, residual toxicity etc (Ekeh et al., 2013).

Powders of six botanical plants were tested for their effectiveness in deterring the developmental stages and reducing the longevity of *C. maculatus* in leguminous grains, viz: *Capsicum nigrum*, *Aframomum melegueta*, *Allium sativum*, *Zingiber officinale*, *Azadiracta indica* and *Ocimum gratissimum*. A synthetic pesticide (pirimiphos methyl) was employed to compare the action with that of natural or medicinal plants. If the action of the medicinal plants to that of synthetic pesticide is comparable up to 50% and above, then the medicinal pesticide will be regarded as fit to replace the synthetic pesticide. The choice of leguminous grains was based on their preference by *C. maculatus*, the high rate of their consumption by households, and their activity with blood cholesterol.

MATERIALS AND METHODS

Procurement of legume seeds

Selected leguminous seeds infested by *C. maculatus* were collected from the market and brought to the laboratory of Department of Zoology and Environmental Biology of University of Nigeria, Nsukka. Cowpea seed (*Vigna unguiculata*), Bambara nut seed (*Vigna subterranea*) and Pigeon pea seed (*Cajanus cajan*) were fumigated for 24 h with no pest synthetic chemical (phostoxin) before the commencement of the experiment in order to kill any insect pest present. The seeds were then exposed for 48 h to get rid of the gas and then sieved with a 2 mm sieve to remove dead insects and exuviate. The seeds were then packaged into polythene bags and later used for the experiment.

Insect culture

The infested seeds were set aside in a plastic container and covered with muslin cloth till the emergence of adults. Healthy adult *C. maculatus* as described by Singh and Pandey (2001) emerged from the container were shifted to other plastic containers and provided clean cowpea seeds, clean bambara nut seeds and clean pigeon pea seeds for oviposition and maintained at 36°C and 56% rh. When oviposition was noticed, the adult *C. maculatus* were removed using 2 mm sieve. The containers with oviposited seeds were left undisturbed until the emergence of adults. Freshly emerged adults of the progeny and subsequent generations were used for the study and for further experiments.

Preparation of plant extract/biopesticides

The plant materials were evaluated for deterrent activity against *C. maculatus*. The parts used and other information are provided in Table 1. The biopesticides used for this study were collected from International Centre for Ethno-medicine and Drug Development (INTERCEDD), Nsukka. The biopesticides were shade-dried, sun and/or oven dried to eliminate all traces of water, after which they were ground into powder, tied in plastic bags and preserved in a refrigerator until needed. All extractions made were powder extraction methods, according to Kawecki (1995) and Ogunwolu and Idowu (1994). A synthetic pesticide was also used as a standard control. The pirimiphos methyl dust used in the experiment was purchased from Zhejiang Linghua, China.

Proximate evaluation of the selected grains

The leguminous grains selected for the study were assessed based on protein, carbohydrate, moisture, fat, ash, crude fibre contents. The percentage levels of these constituents in each grain and the level of influence each have on the pest were ascertained

Phytochemical analysis of plant materials

An electronic balance Metler PC 2000 was used to measure out 5 g each of powdered plant material and mixed in 25 ml of distilled water, boiled at 60°C for 30 min on water bath and then filtered through What man No. 1 filter paper. The filtrate was centrifuged at 2500 rpm for 15 min, the supernatants were discarded and the residues stored in sterile bottles at 5°C (Messina and Slade, 1999) and later used for qualitative phytochemical analysis to ascertain levels of alkaloids, glycosides, saponin, tannins, sugar, steroids, terpenoids, acidic compounds, flavonoids and resins in the botanicals (AOAC, 2000).

Experimental design or procedure

Three leguminous grains were selected for the study, six biopesticides were also used. Each of the biopesticides was used at different concentrations in the three legume grains. Split plot design of six treatments replicated 3 times was adopted in the experiment. The concentrations were 0, 1, 5, 33, 25 and 20% in different jars (Diameter = 0.09 m, $V = 3.69 \times 10^{-3} \text{ m}^3$). Each jar contained 20 g of a particular legume (either *V. Unguiculata*, *V. subterranea* or *C. cajan*), concentration of a particular treatment (either *C. Nigrum*, *A. Melegueta*, *O. Gratissimum*, *A. Sativum*, *A. Indica*, *Z. officinale*), and two pairs of male and female *C. maculatus* and covered with muslin cloth. The control group is in the experimental set up as 0 g and was devoid of treatment. The synthetic pesticide (pirimiphos methyl) used was applied at the rate of 0.1 g per 20 g of each grain.

Table 1. List of experimental plants and parts used against *C. Maculatus*.

Plant	Common name	Family	Part used
<i>Aframomum melegueta</i>	Grain of paradise (<i>Ose oji</i>)	Zingiberaceae	Seed
<i>Capsicum nigrum</i>	Chilli pepper	Solanaceae	Seed
<i>Zingiber officinale</i>	Ginger	Zingibaraceae	Rhizome
<i>Ocimum gratissimum</i>	Scent leaf	Labiataceae	Leaves
<i>Azadiracta indica</i>	Neem	Meliaceae	Leaves
<i>Allium sativa</i>	Garlic	Liliaceae	Bulb

The set up was allowed for 8 weeks and observations were made on alternate days to detect emergence and duration of each developmental stage of *C. maculatus* (egg, larva, pupa and adult).

Data analysis

Mean percentage longevity was determined using descriptive statistics for the six treatments. Differences in treatment means was ascertained using one way analysis of variance (ANOVA). Fisher least significant differences were employed to separate significant treatment means at $P < 0.05$ using the SPSS statistical software package.

RESULTS

There was reduction in the longevity of *C. maculatus* in all the grains studied (Table 2). Longevity of *C. maculatus* was dependent on biopesticide concentration with higher doses deterring the emergence of *C. maculatus* stages. The highest delay in development of *Callosobruchus* was observed with pirimiphos methyl in all the grains sampled. Also the development was recorded only at egg stage, while other developmental stages could not emerge with pirimiphos methyl (Table 2) which include: egg 40.00 ± 0.00 , larva 0.00 ± 0.00 , pupa 0.00 ± 0.00 , adult 0.00 ± 0.00 for *V. unguiculata*, egg 38.00 ± 0.00 , larva 0.00 ± 0.00 , pupa 0.00 ± 0.00 and adult 0.00 ± 0.00 for *C. cajan*, also, egg 37.00 ± 0.00 , larva 0.00 ± 0.00 , pupa 0.00 ± 0.00 and adult 0.00 ± 0.00 for *V. subterranea*. The botanicals used were effective in various concentrations when compared with the control, but the highest delay in development of *C. maculatus* was recorded in *A. sativum* and *C. nigrum* in all the grains at the highest dose rate (Table 2): In *A. sativum* egg stage emerged on the 18.00 ± 0.00 day, larva on the 24.00 ± 0.00 day, pupa on the 36.00 ± 0.00 day and adult on the 40.00 ± 0.00 day on *V. unguiculata*. For *C. nigrum*: In egg stage, it took *C. maculatus* 16.00 ± 0.00 days to emerge, larva stage 24.00 ± 0.00 days, pupa 36.00 ± 0.00 days, and adult 39.00 ± 0.00 days on *V. unguiculata*. In *C. cajan* grain, *A. sativum* at the highest dose rate egg stage of *C. maculatus* took 15.00 days to emerge, larva stage took 22.00 days, pupa stage took 33.00 days and adult stage took 38.00 days to emerge followed by *C. nigrum* at the highest dose rate: egg stage took 12.00

days to emerge, larva stage 22.00 days, pupa stage 33.00 days, adult stage took 36.00 days to emerge while in *V. subterranea* grain, *A. sativum* at the highest dose rate, egg stage took 13.00 days to emerge, larva took 19.00 days, pupa took 33.00 days and adult stage took 37.00 days to emerge, followed by *C. nigrum* at the highest dose rate: egg stage of *C. maculatus* emerged at 10.00 days, larva stage emerged at 16.00 days, pupa stage emerged at 32.00 days and adult stage emerged at 34.00 days. All other botanicals/treatments behaved alike and effective when compared with the control (Table 2). The efficacy and performance of botanicals also showed that *A. sativum* and *C. nigrum* delayed the emergence of *C. maculatus* stages more than all other biopesticides treatments (Table 2).

DISCUSSION

The proximate results of the legume grains used in this study revealed that moisture level in cowpea was approximately similar to that of *C. cajan* and both were significantly different from that of *V. subterranea* (1.2%). The ash, fat and crude fibre percentages in the three legumes used were significantly different from each other. The protein content proportions of *V. unguiculata* differed from that of *V. subterranea* and *C. cajan*. The carbohydrate content of *V. unguiculata* differed significantly from that of *V. subterranea* and *C. cajan*. The physicochemical studies of the six biopesticides used in the study indicated that alkaloids were abundantly (++++) present in *C. nigrum*, *A. sativum* and *Z. officinale*, it was also found to be present in high concentration (+++) in *A. melegueta* and present in very small concentration (+) in *A. indica* and *O. gratissimum*. Similarly, glycosides were found to be abundantly present in *Z. officinale* and *O. gratissimum*, present in high concentration in *A. indica*, *A. melegueta*, *C. nigrum* and *A. sativum*. Saponins were present in high concentration in *O. gratissimum*, moderately (++) present in *A. indica*, present in very small concentration in *C. nigrum* and *A. sativum* and absent (-) in *A. melegueta* and *Z. officinale*. Tannins were found to be abundantly present in *A. melegueta*, moderately present in *A. indica* and *O. gratissimum* and absent in *C. nigrum*, *A. sativum* and *Z. officinale*.

Table 2. Development and longevity study of *C. maculatus* in some selected legumes and biopesticidal activities of the botanicals.

Grain	Biopesticide	Conc. (g)	Egg emergent	Larva emergent	Pupa emergent Days	Adult emergent	P-value
<i>Vigna unguiculata</i>	<i>Aframomum melegueta</i>	0	10.00±0.00	17.00±0.00	28.00±0.00	30.00±0.00	0.01
		0.2	12.00±0.00	18.00±0.00	31.00±0.00	33.00±0.00	0.01
		0.4	12.00±0.00	18.00±0.00	31.00±0.00	33.00±0.00	0.01
		0.6	13.00±0.00	19.00±0.00	32.00±0.00	34.00±0.00	0.01
		0.8	14.00±0.00	20.00±0.00	33.00±0.00	35.00±0.00	0.01
		1.0	15.00±0.00	21.00±0.00	34.00±0.00	36.00±0.00	0.01
	<i>Capsicum nigrum</i>	0	10.00±0.00	17.00±0.00	28.00±0.00	30.00±0.00	0.01
		0.2	12.00±0.00	18.00±0.00	31.00±0.00	33.00±0.00	0.01
		0.4	12.00±0.00	18.00±0.00	31.00±0.00	33.00±0.00	0.01
		0.6	14.00±0.00	20.00±0.00	32.00±0.00	34.00±0.00	0.01
		0.8	15.00±0.00	21.00±0.00	33.00±0.00	35.00±0.00	0.01
		1.0	16.00±0.00	24.00±0.00	36.00±0.00	39.00±0.00	0.01
	<i>Allium sativum</i>	0	10.00±0.00	17.00±0.00	28.00±0.00	30.00±0.00	0.01
		0.2	13.00±0.00	19.00±0.00	33.00±0.00	34.00±0.00	0.01
		0.4	13.00±0.00	19.00±0.00	33.00±0.00	34.00±0.00	0.01
		0.6	14.00±0.00	19.00±0.00	33.00±0.00	34.00±0.00	0.01
		0.8	15.00±0.00	21.00±0.00	35.00±0.00	36.00±0.00	0.01
		1.0	18.00±0.00	24.00±0.00	36.00±0.00	40.00±0.00	0.01
<i>Zingiber officinale</i>	0	10.00±0.00	17.00±0.00	28.00±0.00	30.00±0.00	0.01	
	0.2	13.00±0.00	19.00±0.00	33.00±0.00	34.00±0.00	0.01	
	0.4	13.00±0.00	19.00±0.00	33.00±0.00	34.00±0.00	0.01	
	0.6	14.00±0.00	19.00±0.00	33.00±0.00	34.00±0.00	0.01	
	0.8	15.00±0.00	21.00±0.00	35.00±0.00	36.00±0.00	0.01	
	1.0	16.00±0.00	22.00±0.00	36.00±0.00	38.00±0.00	0.01	
<i>Azadiracta indica</i>	0	10.00±0.00	17.00±0.00	28.00±0.00	30.00±0.00	0.01	
	0.2	12.00±0.00	18.00±0.00	31.00±0.00	33.00±0.00	0.01	
	0.4	12.00±0.00	18.00±0.00	31.00±0.00	33.00±0.00	0.01	
	0.6	13.00±0.00	19.00±0.00	32.00±0.00	34.00±0.00	0.01	
	0.8	14.00±0.00	20.00±0.00	33.00±0.00	35.00±0.00	0.01	
	1.0	15.00±0.00	21.00±0.00	34.00±0.00	36.00±0.00	0.01	

Table 2. Cont'd.

	0	10.00±0.00	17.00±0.00	28.00±0.00	30.00±0.00	0.01
	0.2	12.00±0.00	18.00±0.00	31.00±0.00	33.00±0.00	0.01
<i>Ocimum</i>	0.4	12.00±0.00	18.00±0.00	31.00±0.00	33.00±0.00	0.01
<i>gratissimum</i>	0.6	13.00±0.00	19.00±0.00	32.00±0.00	34.00±0.00	0.01
	0.8	14.00±0.00	20.00±0.00	33.00±0.00	35.00±0.00	0.01
	1.0	15.00±0.00	21.00±0.00	34.00±0.00	36.00±0.00	0.01
Pirimiphos methyl	0.1	28.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.01
	0	5.00±0.00	17.00±0.00	25.00±0.00	27.00±0.00	0.01
	0.2	8.00±0.00	18.00±0.00	28.00±0.00	30.00±0.00	0.01
<i>Aframomum</i>	0.4	8.00±0.00	18.00±0.00	29.00±0.00	30.00±0.00	0.01
<i>melegueta</i>	0.6	9.00±0.00	19.00±0.00	30.00±0.00	31.00±0.00	0.01
	0.8	10.00±0.00	20.00±0.00	31.00±0.00	33.00±0.00	0.01
	1.0	11.00±0.00	21.00±0.00	32.00±0.00	34.00±0.00	0.01
	0	5.00±0.00	12.00±0.00	25.00±0.00	27.00±0.00	0.01
	0.2	6.00±0.00	15.00±0.00	28.00±0.00	30.00±0.00	0.01
<i>Capsicum</i>	0.4	6.00±0.00	15.00±0.00	28.00±0.00	30.00±0.00	0.01
<i>nigrum</i>	0.6	6.00±0.00	16.00±0.00	29.00±0.00	30.00±0.00	0.01
	0.8	8.00±0.00	17.00±0.00	30.00±0.00	31.00±0.00	0.01
<i>Cajanus cajan</i>	1.0	12.00±0.00	22.00±0.00	33.00±0.00	36.00±0.00	0.01
	0	5.00±0.00	12.00±0.00	25.00±0.00	27.00±0.00	0.01
	0.2	9.00±0.00	13.00±0.00	30.00±0.00	31.00±0.00	0.01
<i>Allium sativum</i>	0.4	9.00±0.00	13.00±0.00	30.00±0.00	31.00±0.00	0.01
	0.6	10.00±0.00	14.00±0.00	31.00±0.00	32.00±0.00	0.01
	0.8	11.00±0.00	15.00±0.00	32.00±0.00	33.00±0.00	0.01
	1.0	15.00±0.00	22.00±0.00	33.00±0.00	38.00±0.00	0.01
	0	5.00±0.00	12.00±0.00	25.00±0.00	27.00±0.00	0.01
	0.2	8.00±0.00	15.00±0.00	30.00±0.00	31.00±0.00	0.01
<i>Zingiber</i>	0.4	8.00±0.00	15.00±0.00	30.00±0.00	31.00±0.00	0.01
<i>officinale</i>	0.6	9.00±0.00	16.00±0.00	31.00±0.00	32.00±0.00	0.01
	0.8	10.00±0.00	17.00±0.00	32.00±0.00	33.00±0.00	0.01
	1.0	11.00±0.00	18.00±0.00	33.00±0.00	34.00±0.00	0.01

Table 2 cont'd

		0	5.00±0.00	12.00±0.00	25.00±0.00	27.00±0.00	0.01
		0.2	6.00±0.00	13.00±0.00	28.00±0.00	30.00±0.00	0.01
	<i>Azadiracta</i>	0.4	6.00±0.00	13.00±0.00	28.00±0.00	30.00±0.00	0.01
	<i>indica</i>	0.6	7.00±0.00	14.00±0.00	29.00±0.00	31.00±0.00	0.01
		0.8	8.00±0.00	15.00±0.00	30.00±0.00	32.00±0.00	0.01
		1.0	9.00±0.00	16.00±0.00	31.00±0.00	33.00±0.00	0.01
		0	5.00±0.00	12.00±0.00	25.00±0.00	27.00±0.00	0.01
		0.2	6.00±0.00	13.00±0.00	28.00±0.00	30.00±0.00	0.01
	<i>Ocimum</i>	0.4	6.00±0.00	13.00±0.00	28.00±0.00	30.00±0.00	0.01
	<i>gratissimum</i>	0.6	7.00±0.00	14.00±0.00	29.00±0.00	31.00±0.00	0.01
		0.8	8.00±0.00	15.00±0.00	30.00±0.00	32.00±0.00	0.01
		1.0	9.00±0.00	16.00±0.00	31.00±0.00	33.00±0.00	0.01
	Pirimiphos methyl	0.1	26.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.01
		0	5.00±0.00	12.00±0.00	25.00±0.00	27.00±0.00	0.01
		0.2	9.00±0.00	16.00±0.00	29.00±0.00	31.00±0.00	0.01
	<i>Aframomum</i>	0.4	9.00±0.00	16.00±0.00	29.00±0.00	31.00±0.00	0.01
	<i>melegueta</i>	0.6	10.00±0.00	17.00±0.00	30.00±0.00	32.00±0.00	0.01
		0.8	11.00±0.00	18.00±0.00	31.00±0.00	33.00±0.00	0.01
		1.0	12.00±0.00	19.00±0.00	32.00±0.00	34.00±0.00	0.01
		0	5.00±0.00	12.00±0.00	25.00±0.00	27.00±0.00	0.01
		0.2	6.00±0.00	13.00±0.00	28.00±0.00	30.00±0.00	0.01
<i>Vigna subterranea</i>	<i>Capsicum</i>	0.4	6.00±0.00	13.00±0.00	29.00±0.00	31.00±0.00	0.01
	<i>nigrum</i>	0.6	7.00±0.00	14.00±0.00	30.00±0.00	32.00±0.00	0.01
		0.8	8.00±0.00	15.00±0.00	31.00±0.00	33.00±0.00	0.01
		1.0	10.00±0.00	16.00±0.00	32.00±0.00	34.00±0.00	0.01
		0	5.00±0.00	12.00±0.00	25.00±0.00	27.00±0.00	0.01
		0.2	10.00±0.00	16.00±0.00	30.00±0.00	32.00±0.00	0.01
	<i>Allium sativum</i>	0.4	10.00±0.00	16.00±0.00	30.00±0.00	32.00±0.00	0.01
		0.6	11.00±0.00	17.00±0.00	31.00±0.00	33.00±0.00	0.01
		0.8	12.00±0.00	18.00±0.00	32.00±0.00	34.00±0.00	0.01
		1.0	13.00±0.00	19.00±0.00	33.00±0.00	37.00±0.00	0.01

Table 2. Cont'd.

	0	5.00±0.00	12.00±0.00	25.00±0.00	27.00±0.00	0.01
	0.2	9.00±0.00	16.00±0.00	29.00±0.00	31.00±0.00	0.01
<i>Zingiber officinale</i>	0.4	9.00±0.00	16.00±0.00	30.00±0.00	31.00±0.00	0.01
	0.6	10.00±0.00	17.00±0.00	31.00±0.00	32.00±0.00	0.01
	0.8	11.00±0.00	18.00±0.00	32.00±0.00	33.00±0.00	0.01
	1.0	12.00±0.00	19.00±0.00	33.00±0.00	34.00±0.00	0.01
	0	5.00±0.00	12.00±0.00	25.00±0.00	27.00±0.00	0.01
	0.2	6.00±0.00	13.00±0.00	28.00±0.00	30.00±0.00	0.01
<i>Azadiracta indica</i>	0.4	6.00±0.00	13.00±0.00	28.00±0.00	30.00±0.00	0.01
	0.6	7.00±0.00	14.00±0.00	29.00±0.00	31.00±0.00	0.01
	0.8	8.00±0.00	15.00±0.00	30.00±0.00	32.00±0.00	0.01
	1.0	9.00±0.00	16.00±0.00	31.00±0.00	33.00±0.00	0.01
	0	5.00±0.00	12.00±0.00	25.00±0.00	27.00±0.00	0.01
	0.2	6.00±0.00	13.00±0.00	28.00±0.00	30.00±0.00	0.01
<i>Ocimum gratissimum</i>	0.4	6.00±0.00	13.00±0.00	28.00±0.00	30.00±0.00	0.01
	0.6	7.00±0.00	14.00±0.00	29.00±0.00	31.00±0.00	0.01
	0.8	8.00±0.00	15.00±0.00	30.00±0.00	32.00±0.00	0.01
	1.0	9.00±0.00	16.00±0.00	31.00±0.00	33.00±0.00	0.01
Pirimiphos methyl	0.1	24.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.01
P-value		0.01	0.01	0.01	0.01	

Furthermore, reducing sugar was present in high concentration in *C. nigrum*, moderately present in *A. melegueta* and *O. gratissimum*, present in very small concentration in *A. indica*, *A. sativum* and *Z. officinale*. Steroids and terpenoids were abundantly present in *A. melegueta*, highly present in *A. sativum*, moderately present in *Z. officinale* and present in small concentration in *A. indica*, *C. nigrum* and *O. gratissimum*. Acidic compounds were not present in the biopesticides

studied, except in *A. sativum* where it was present in very small concentration. Flavonoids were abundantly present in *A. melegueta*, present in high concentration in *O. gratissimum*, moderately present in *Z. officinale*, present in very small concentration in *A. indica* and *C. nigrum* and was absent in *A. sativum*. Lastly, resins were abundantly present in *C. nigrum*, present in very small concentration in *A. indica*, *A. melegueta*, *Z. officinale* and *O. gratissimum* and was absent in

A. sativum. From the study, it was discovered that biopesticide treatments were effective in delaying the developmental stage emergence in *C. maculatus* especially at the highest dose rate (1.0 g). The statement agreed with the findings of Rahman and Talukder (2002) who observed the effective-ness of *Thymus vulgaris*, *Santolia chamaecyparissus* and *Anagyris foetida* powder in delaying stage emergence of *C. chinensis*. They submitted that stage emergence was

Table 3. Efficacy and performance of biopesticides concentrations on development/longevity of *callosobruchus maculatus* in grains.

Stage	Biopesticides	Grains			
		<i>V. unguiculata</i>	<i>C. cajan</i>	<i>V. subterranea</i>	
Egg	0	<i>Aframomum melegueta</i>	11.00 ^a	5.00 ^b	6.00 ^c
		<i>Capsicum nigrum</i>	11.00 ^a	5.00 ^b	6.00 ^c
		<i>Allium sativum</i>	11.00 ^a	5.00 ^b	6.00 ^c
		<i>Zingiber officinale</i>	11.00 ^a	5.00 ^b	6.00 ^c
		<i>Azadiracta indica</i>	11.00 ^a	5.00 ^b	6.00 ^c
		<i>Ocimum gratissimum</i>	11.00 ^a	5.00 ^b	6.00 ^c
	0.2	<i>Aframomum melegueta</i>	12.00 ^a	6.00 ^b	7.00 ^c
		<i>Capsicum nigrum</i>	13.00 ^a	8.00 ^b	8.00 ^b
		<i>Allium sativum</i>	13.00 ^a	9.00 ^b	10.00 ^c
		<i>Zingiber officinale</i>	13.00 ^a	8.00 ^b	9.00 ^c
		<i>Azadiracta indica</i>	12.00 ^a	6.00 ^b	6.00 ^b
		<i>Ocimum gratissimum</i>	12.00 ^a	6.00 ^b	6.00 ^b
	0.4	<i>Aframomum melegueta</i>	12.00 ^a	6.00 ^b	9.00 ^c
		<i>Capsicum nigrum</i>	13.00 ^a	8.00 ^b	9.00 ^b
		<i>Allium sativum</i>	13.00 ^a	9.00 ^b	10.00 ^c
		<i>Zingiber officinale</i>	13.00 ^a	8.00 ^b	9.00 ^c
		<i>Azadiracta indica</i>	12.00 ^a	6.00 ^b	6.00 ^b
		<i>Ocimum gratissimum</i>	12.00 ^a	6.00 ^b	6.00 ^b
	0.6	<i>Aframomum melegueta</i>	12.00 ^a	6.00 ^b	7.00 ^c
		<i>Capsicum nigrum</i>	12.00 ^a	8.00 ^b	9.00 ^b
		<i>Allium sativum</i>	13.00 ^a	9.00 ^b	10.00 ^c
		<i>Zingiber officinale</i>	13.00 ^a	8.00 ^b	9.00 ^c
		<i>Azadiracta indica</i>	12.00 ^a	6.00 ^b	6.00 ^b
		<i>Ocimum gratissimum</i>	12.00 ^a	6.00 ^b	6.00 ^b
	0.8	<i>Aframomum melegueta</i>	10.00 ^a	8.00 ^a	8.00 ^a
		<i>Capsicum nigrum</i>	12.00 ^a	10.00 ^a	10.00 ^a
		<i>Allium sativum</i>	13.00 ^a	11.00 ^a	11.00 ^a
		<i>Zingiber officinale</i>	10.00 ^a	10.00 ^a	9.00 ^a
		<i>Azadiracta indica</i>	9.00 ^a	9.00 ^a	9.00 ^a
		<i>Ocimum gratissimum</i>	9.00 ^a	9.00 ^a	9.00 ^a
1.0	<i>Aframomum melegueta</i>	10.00 ^a	10.00 ^a	10.00 ^a	
	<i>Capsicum nigrum</i>	16.00 ^a	14.00 ^a	13.00 ^a	
	<i>Allium sativum</i>	18.00 ^a	16.00 ^a	14.00 ^a	
	<i>Zingiber officinale</i>	10.00 ^a	10.00 ^a	10.00 ^a	
	<i>Azadiracta indica</i>	10.00 ^a	10.00 ^a	9.00 ^a	
	<i>Ocimum gratissimum</i>	11.00 ^a	10.00 ^a	10.00 ^a	
Larva	0	<i>Aframomum melegueta</i>	17.00 ^a	12.00 ^b	12.00 ^b
		<i>Capsicum nigrum</i>	17.00 ^a	12.00 ^b	12.00 ^b
		<i>Allium sativum</i>	17.00 ^a	12.00 ^b	12.00 ^b
		<i>Zingiber officinale</i>	17.00 ^a	12.00 ^b	12.00 ^b
		<i>Azadiracta indica</i>	17.00 ^a	12.00 ^b	12.00 ^b
		<i>Ocimum gratissimum</i>	17.00 ^a	12.00 ^b	12.00 ^b

Table 2. Cont'd.

0.2	<i>Aframomum melegueta</i>	18.00 ^a	15.00 ^b	16.00 ^c	
	<i>Capsicum nigrum</i>	18.00 ^a	16.00 ^b	15.00 ^b	
	<i>Allium sativum</i>	19.00 ^a	18.00 ^b	16.00 ^c	
	<i>Zingiber officinale</i>	17.00 ^a	16.00 ^b	15.00 ^c	
	<i>Azadiracta indica</i>	16.00 ^a	13.00 ^b	13.00 ^b	
	<i>Ocimum gratissimum</i>	16.00 ^a	13.00 ^b	13.00 ^b	
0.4	<i>Aframomum melegueta</i>	18.00 ^a	15.00 ^b	16.00 ^c	
	<i>Capsicum nigrum</i>	18.00 ^a	16.00 ^b	16.00 ^b	
	<i>Allium sativum</i>	19.00 ^a	17.00 ^b	16.00 ^c	
	<i>Zingiber officinale</i>	19.00 ^a	15.00 ^b	16.00 ^c	
	<i>Azadiracta indica</i>	18.00 ^a	13.00 ^b	13.00 ^b	
	<i>Ocimum gratissimum</i>	18.00 ^a	13.00 ^b	13.00 ^b	
0.6	<i>Aframomum melegueta</i>	18.00 ^a	15.00 ^b	16.00 ^c	
	<i>Capsicum nigrum</i>	18.00 ^a	16.00 ^b	14.00 ^b	
	<i>Allium sativum</i>	19.00 ^a	17.00 ^b	16.00 ^c	
	<i>Zingiber officinale</i>	19.00 ^a	15.00 ^b	14.00 ^c	
	<i>Azadiracta indica</i>	18.00 ^a	13.00 ^b	13.00 ^b	
	<i>Ocimum gratissimum</i>	18.00 ^a	13.00 ^b	13.00 ^b	
0.8	<i>Aframomum melegueta</i>	15.00 ^a	15.00 ^a	15.00 ^a	
	<i>Capsicum nigrum</i>	18.00 ^a	16.00 ^a	15.00 ^a	
	<i>Allium sativum</i>	19.00 ^a	18.00 ^a	16.00 ^a	
	<i>Zingiber officinale</i>	18.00 ^a	15.00 ^a	15.00 ^a	
	<i>Azadiracta indica</i>	15.00 ^a	15.00 ^a	15.00 ^a	
	<i>Ocimum gratissimum</i>	15.00 ^a	15.00 ^a	15.00 ^a	
1.0	<i>Aframomum melegueta</i>	16.00 ^a	16.00 ^a	16.00 ^a	
	<i>Capsicum nigrum</i>	19.00 ^a	17.00 ^a	16.00 ^a	
	<i>Allium sativum</i>	20.00 ^a	19.00 ^a	17.00 ^a	
	<i>Zingiber officinale</i>	18.00 ^a	16.00 ^a	16.00 ^a	
	<i>Azadiracta indica</i>	16.00 ^a	16.00 ^a	15.00 ^a	
	<i>Ocimum gratissimum</i>	17.00 ^a	16.00 ^a	16.00 ^a	
Pupa	<i>Aframomum melegueta</i>	30.00 ^a	28.00 ^b	27.00 ^c	
	<i>Capsicum nigrum</i>	30.00 ^a	27.00 ^b	27.00 ^b	
	<i>Allium sativum</i>	31.00 ^a	28.00 ^b	27.00 ^c	
	<i>Zingiber officinale</i>	31.00 ^a	28.00 ^b	27.00 ^c	
	<i>Azadiracta indica</i>	30.00 ^a	27.00 ^b	27.00 ^b	
	<i>Ocimum gratissimum</i>	30.00 ^a	27.00 ^b	27.00 ^b	
	0.2	<i>Aframomum melegueta</i>	31.00 ^a	28.00 ^b	29.00 ^c
		<i>Capsicum nigrum</i>	34.00 ^a	32.00 ^b	31.00 ^b
		<i>Allium sativum</i>	36.00 ^a	34.00 ^b	33.00 ^b
		<i>Zingiber officinale</i>	33.00 ^a	30.00 ^b	29.00 ^c
		<i>Azadiracta indica</i>	31.00 ^a	29.00 ^b	28.00 ^b
		<i>Ocimum gratissimum</i>	31.00 ^a	28.00 ^b	27.00 ^b

Table 3. Cont'd.

		<i>Aframomum melegueta</i>	31.00 ^a	29.00 ^b	29.00 ^b
		<i>Capsicum nigrum</i>	34.00 ^a	32.00 ^b	31.00 ^c
	0.4	<i>Allium sativum</i>	36.00 ^a	35.00 ^b	34.00 ^b
		<i>Zingiber officinale</i>	33.00 ^a	31.00 ^b	30.00 ^b
		<i>Azadiracta indica</i>	31.00 ^a	29.00 ^b	28.00 ^b
		<i>Ocimum gratissimum</i>	31.00 ^a	30.00 ^b	28.00 ^b
		<i>Aframomum melegueta</i>	31.00 ^a	29.00 ^b	29.00 ^b
		<i>Capsicum nigrum</i>	36.00 ^a	34.00 ^b	33.00 ^c
	0.6	<i>Allium sativum</i>	38.00 ^a	36.00 ^b	34.00 ^b
		<i>Zingiber officinale</i>	33.00 ^a	30.00 ^b	30.00 ^b
		<i>Azadiracta indica</i>	31.00 ^a	29.00 ^b	28.00 ^b
		<i>Ocimum gratissimum</i>	31.00 ^a	30.00 ^b	29.00 ^b
		<i>Aframomum melegueta</i>	30.00 ^a	30.00 ^a	30.00 ^a
		<i>Capsicum nigrum</i>	36.00 ^a	34.00 ^a	33.00 ^a
	0.8	<i>Allium sativum</i>	38.00 ^a	37.00 ^a	35.00 ^a
		<i>Zingiber officinale</i>	34.00 ^a	30.00 ^a	30.00 ^a
		<i>Azadiracta indica</i>	33.00 ^a	31.00 ^a	30.00 ^a
		<i>Ocimum gratissimum</i>	33.00 ^a	32.00 ^a	31.00 ^a
		<i>Aframomum melegueta</i>	31.00 ^a	31.00 ^a	30.00 ^a
		<i>Capsicum nigrum</i>	36.00 ^a	36.00 ^a	34.00 ^a
	1.0	<i>Allium sativum</i>	38.00 ^a	37.00 ^a	35.00 ^a
		<i>Zingiber officinale</i>	35.00 ^a	32.00 ^a	31.00 ^a
		<i>Azadiracta indica</i>	33.00 ^a	31.00 ^a	31.00 ^a
		<i>Ocimum gratissimum</i>	34.00 ^a	32.00 ^a	31.00 ^a
		<i>Aframomum melegueta</i>	32.00 ^a	29.00 ^b	29.00 ^b
		<i>Capsicum nigrum</i>	32.00 ^a	29.00 ^b	29.00 ^b
	0	<i>Allium sativum</i>	32.00 ^a	29.00 ^b	29.00 ^b
		<i>Zingiber officinale</i>	32.00 ^a	29.00 ^b	29.00 ^b
		<i>Azadiracta indica</i>	32.00 ^a	29.00 ^b	29.00 ^b
		<i>Ocimum gratissimum</i>	32.00 ^a	29.00 ^b	29.00 ^b
		<i>Aframomum melegueta</i>	33.00 ^a	30.00 ^b	31.00 ^c
		<i>Capsicum nigrum</i>	33.00 ^a	30.00 ^b	30.00 ^b
	0.2	<i>Allium sativum</i>	34.00 ^a	31.00 ^b	32.00 ^c
Adult		<i>Zingiber officinale</i>	34.00 ^a	31.00 ^b	31.00 ^c
		<i>Azadiracta indica</i>	33.00 ^a	30.00 ^b	30.00 ^b
		<i>Ocimum gratissimum</i>	33.00 ^a	30.00 ^b	30.00 ^b
		<i>Aframomum melegueta</i>	33.00 ^a	30.00 ^b	31.00 ^c
		<i>Capsicum nigrum</i>	33.00 ^a	30.00 ^b	31.00 ^c
	0.4	<i>Allium sativum</i>	34.00 ^a	31.00 ^b	32.00 ^c
		<i>Zingiber officinale</i>	34.00 ^a	31.00 ^b	31.00 ^b
		<i>Azadiracta indica</i>	33.00 ^a	30.00 ^b	30.00 ^b
		<i>Ocimum gratissimum</i>	33.00 ^a	30.00 ^b	30.00 ^b

Table 3. Cont'd.

0.6	<i>Aframomum melegueta</i>	33.00 ^a	30.00 ^b	31.00 ^c
	<i>Capsicum nigrum</i>	33.00 ^a	30.00 ^b	31.00 ^c
	<i>Allium sativum</i>	34.00 ^a	31.00 ^b	32.00 ^c
	<i>Zingiber officinale</i>	34.00 ^a	31.00 ^b	31.00 ^b
	<i>Azadiracta indica</i>	33.00 ^a	30.00 ^b	30.00 ^b
	<i>Ocimum gratissimum</i>	33.00 ^a	30.00 ^b	30.00 ^b
0.8	<i>Aframomum melegueta</i>	33.00 ^a	33.00 ^a	33.00 ^a
	<i>Capsicum nigrum</i>	33.00 ^a	33.00 ^a	33.00 ^a
	<i>Allium sativum</i>	33.00 ^a	33.00 ^a	33.00 ^a
	<i>Zingiber officinale</i>	33.00 ^a	33.00 ^a	33.00 ^a
	<i>Azadiracta indica</i>	33.00 ^a	33.00 ^a	33.00 ^a
	<i>Ocimum gratissimum</i>	33.00 ^a	33.00 ^a	33.00 ^a
1.0	<i>Aframomum melegueta</i>	34.00 ^a	34.00 ^a	34.00 ^a
	<i>Capsicum nigrum</i>	34.00 ^a	34.00 ^a	34.00 ^a
	<i>Allium sativum</i>	34.00 ^a	34.00 ^a	34.00 ^a
	<i>Zingiber officinale</i>	34.00 ^a	34.00 ^a	34.00 ^a
	<i>Azadiracta indica</i>	34.00 ^a	34.00 ^a	34.00 ^a
	<i>Ocimum gratissimum</i>	34.00 ^a	34.00 ^a	34.00 ^a

LSD ($p \leq 0.05$) 0.000 Superscript with the same letter not significant, Superscript with different letter significant. The proximate result of the legume grains used in this study revealed the percentage levels

Table 4. Proximate analysis of leguminous grains.

Nutrient	% Composition		
	<i>Vigna unguiculata</i>	<i>Vigna subterranea</i>	<i>Cajanus cajan</i>
Moisture	6.95	1.2	8.1
Ash	4.3	3.65	3.35
Fat	5.5	6.5	2.5
Crude fibre	2.15	1.83	1.68
Protein	24.44	22.60	21.08
Carbohydrate	56.66	64.22	63.29

delayed following the activities of the treatments used. The effectiveness of biopesticides used in this study was prominent with *A. sativum* where the activities were highest in delaying stage emergence of *C. maculatus* in the three legumes studied. The effectiveness of this bulb powder may be attributed to the presence of different bioactive agents present in them. Mulatu and Gebremedhin (2000) showed that 2.5% powdered seed of *A. indica* were toxic to *C. maculatus* which was similar to the finding in this study.

Mulatu and Gebremedlin (2000) reported that eucalyptus seed powder treatment delayed the emerging adult of *C. maculatus* and when emerged caused the death. Higher doses of *Vittellaria paradoxa* plant seed powder (7.5 and 10.0% w/w) inhibited larva and adult stages of *C. maculatus*. This statement is similar to the

present study where higher doses of treatment used deterred the emergence of larva and adult stages. The development and emergence of *C. maculatus* were found to vary significantly in the various legumes with different doses of the powdered biopesticides used. *Callosobruchus* females generally prefer smooth seed varieties for oviposition (Haines, 1991). The delay of *C. maculatus* stage emergence was higher in treated *V. unguiculata* than other treated legumes used. This can be attributed to the fact that cowpea is a preferred host to *C. maculatus* than other legumes probably because of the nutrient level of the grain and nature of the seed endosperm (Creadland et al., 1986; Utida, 1972).

Proximate studies revealed that legumes contain high concentration of protein, carbohydrates and dietary fibre and make important contributions to human diet in many

Table 5. Phytochemical composition of varied botanicals studied for their biopesticidal activities.

Parameter	<i>Azadiracta indica</i>	<i>Aframomum melegueta</i>	<i>Capsicum nigrum</i>	<i>Allium sativum</i>	<i>Zingiber officinale</i>	<i>Ocimum gratissimum</i>
Alkaloids	+	+++	++++	++++	++++	+
Glycosides	+++	+++	+++	+++	++++	++++
Saponins	++	-	+	+	-	+++
Tannins	++	++++	-	-	-	++
Reducing sugar	+	++	+++	+	+	++
Steroids	+	++++	+	+++	++	+
Terpenoids	+	++++	+	+++	++	+
Acidic compounds	-	-	-	+	-	-
Flavonoids	+	++++	+	-	++	+++
Resins.	+	+	++++	-	+	+

Not present; + Present in very small concentration; ++present in moderately high concentration; +++present in very high concentration; ++++Abundantly present.

countries (Bressani, 1993). The present work agrees with this statement where the proximate result revealed the percentage levels of nutrient constituents in leguminous grains studied and it was seen that they contained high percentage of protein and carbohydrate. Bressani (1993) revealed that the nutritional value of grain legumes includes high protein and lysine content which allow legumes to serve as excellent protein supplement to cereal grains. The health related value of legume includes their positive effect on blood cholesterol and glucose levels (Van and de Rooy, 1998; Lale, 2002) possibly through the dietary fibre present in them. The moisture percentages of legumes in the present work agreed with the work of Leeds (1982). The protein level of the legumes seeds studied differed with the report of Leeds (1982) who worked with Guar gum seed. It also differed with Majeed et al. (2006) who worked with lupin seed, but the differences in the protein proportion of these legumes were attributed to variations in the seed types and processing method employed. The action and effectiveness of pirimiphos methyl (synthetic pesticides) has long been established (Singh, 2011; Ekeh et al., 2013), but the hazardous effect which they exhibit on both man, his animals and environment calls for a substitute which is safe and environmentally friendly. Following the findings, it could be deduced that *A. sativum* and *Capsicum nigrum* were very effective in discouraging or delaying emergence of *C. maculatus* development, and so are suggested to replace (pirimiphos methyl) chemical pesticide in longevity studies.

Conflict of interest

The authors declare that they have no conflict of interest to disclose.

REFERENCES

Bagheri-Zenouz E (1996). Pests of stored products and their control

- methods (Injurious Coleopteran of food and industrial products). I. Sepehre publishers. Tehran. P 309.
- Bressani R (1993). Grain quality of common beans. Food Revolution Int. 9:217-297.
- Creadland PF, Dick KM, Wright AW (1986) . Relationship between larval density, adult size and egg production in the cowpea seed beetle *Callosobruchus maculatus*. Ecol. Entomol. 11(1):41-50.
- Ekeh FN, Onah IE, Atama CI, Ivoke N, Eyo JE (2013). Effectiveness of Botanical powders against *Callosobruchus maculatus* (Coleoptera:Bruchidae) in some stored leguminous grains under laboratory conditions. Afr. J. Biotechnol. 12(12):1384-1391.
- Ekeh FN, Odo GE, Ivoke N, Ubani CS, Nweze NO, Agwu EJ, Osayi EE, Haruna AS (2014). Evaluations on the efficacy of some biopesticidal powders on the Nataly (Birth rate) of *Callosobruchus maculatus* (F) (Coleoptera:Bruchidae) in some leguminous grains. Int. J. Sci. Eng. Res. 5(2):646-657.
- Haines CP (1991). Insects and Arachnids of tropical stored products: their biology and identification. A training manual 2nd Ed. Natural resources institute Hobbs Southampton, UK.
- Kawecki TJ (1995). Adaptive plasticity of egg size in response to competition in the cowpea weevil, *Callosobruchus maculatus* (Coleoptera: Bruchidae) Oecologia 102:81-85.
- Lale NES (2002). Seed powder formulations. Stored Product Entomology and Acarology in Tropical Africa. Mole Publications. 204pp.
- Leeds AR (1982). Legumes and gastrointestinal function in relation to diet for diabetics. J. Plant Food 4:23-27.
- Majeed BA, Rashed AA, Mohammed EA, Amro BH, Elfadil EB (2006). Proximate composition, antinutritional factors and protein fractions of Guar gum seeds as influenced by processing treatments. Pak. J. Nutr. 5(5):481-484.
- Messina F, Slade A (1999). Expression of a life-history trade-off in a seed beetle depends on environmental context. Physiol. Entomol. 24:358-363.
- Mulatu B, Gebremedhin T (2000). Oviposition deterrent and toxic effects of various botanicals on the Adzuki bean beetle, *Callosobruchus chinensis* (L). Insect Sci. Appl. 20(1):33-38.
- Ogunwolu O, Idowu O (1994). Potential of powered *Zanthoxylum zanthoxyloides* (Rutaceae) root bark and *Azadiractha indica* (meliceae) seed for control of the cowpea seed bruchid, *Callosobruchus maculatus* (Bruchidae) in Nigeria. J. Afr. Zool. 108(8):521-528
- Rahman A, Talukder FA (2002). Bioefficacy of some plant derivatives that protect grain against the pulse beetle *Callosobruchus maculatus*, Bangladesh Agricultural University. Mymensingh 2202
- Right-Assia AF, Khehl MA, Medjdoub-Bensaad F, Right K (2010). Efficacy of oils and powders of some medicinal plants in biological control of the pea weevil (*Callosobruchus chinensis*. L.). Afr. J. Agric. Res. 5(12):1474-1481.

- Singh R (2011). Bioecological studies and control of pulse beetle *Callosobruchus chinensis* (Coleoptera:bruchidae) on cowpea seeds. *Adv. Appl. Sci. Res.* 2(2):295-302.
- Singh VN, Pandey ND (2001). Growth and development of *Callosobruchus chinensis* Linn on different varieties. *Indian J. Entomol.* 63(2):182-185.
- Utida S (1972). Density dependent polymorphism in the adult of *Callosobruchus maculatus* (Coleoptera; Bruchidae). *J. Stored Prod. Res.* 8:111-126.
- Van Hius A, de Rooy M (1998). The effect of leguminous plant species on *Callosobruchus maculatus* (Coleoptera: Bruchidae) and its parasitoid *Uscana lariophaga* (Hymenoptera: Trichogrammatidae). *Bull. Entomol. Res.* 88:93-99.