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Oviposition Deterrence and Other Biological Influences of Aqueous Leaves Extracts of Neem, Colocasia and Their Mixtures Alone or Combined with Gamma Radiation to Reduce the Risk of the Potato Tubermoth, *Phthorimaea operculella* (Zeller)

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Abstract: Aqueous extracts of leaves of neem, colocasia and their mixtures reduced percentage infestation of PTM. in field and also the number of survived larvae, pupation and adult emergence percentages. Mixtures of neem and colocasia gave good results followed by neem alone and finally colocasia extract. Oviposition deterrence (ODI) and percentage of sterility were also affected and 100% oviposition deterrence was occurred in concentrations 4.0, and 2.0 in case of treatment with neem alone and treatment with mixtures of neem and colocasia, respectively. Irradiation of full grown pupae at the dose level 50 and 100Gy increased the efficiency of the extracts and the best effect was obtained when mixtures of neem colocasia were used besides 100Gy of gamma radiation, where percent sterility and percent ODI was increased.

Key words: Neem, colocasia, gamma radiation, *Phthorimaea operculella*

Introduction

Potato, *Solanum tuberosum* L. is a crop of international importance for consumers everywhere in the world. Potato tubermoth *Phthorimaea operculella* (Zeller) (PTM) is considered among the most important and destructive potato insect pests. It commonly causes severe direct damage to potato plants in the field and to the yielded tubers in the store and allowing plant diseases to occur. (El-Sinary, 1995; Mariy *et al.,* 1999 and Daoud *et al.,* 1999). The extensive use of chemical control has led to many problems such as the presence of undesirable residues, which are considered as potential health hazards, development of insect resistance or tolerance to chemical and chemicals fumigations fail to kill the larvae that live inside the stored tubers (Haiba, 1990; Ali, 1993 and Doss *et al.,* 1994).

In recent years, there has been an increased interest in natural plant-derived materials as alternative pesticides to conventional, broad-spectrum toxicants. Neem extracts have a wide range of effects against insect pests, including repellence, feeding, oviposition deterrence, toxicity, sterility and growth regulatory-activity (Jacobson, 1989; Schmutterer, 1990, 1995; Ascher, 1993; Van Randen and Roitberg, 1996).

The Egyptian colocasia, *Colcasia antiquorum* is belonging to family Aracae and colocasia leaves are huge and resemble an elephant's ear. This plant show toxic effect to insects (El-Samea, 1990).

The aim of this research is to investigate the effect of aqueous extracts of neem and colocasia and their mixtures on reducing the risk of the potato tubermoth of *Phthorimaea operculella* (Zeller) in field conditions and to determine the oviposition deterrence indices and sterility percentage of their aqueous extracts alone or combined with gamma radiation on the adult females of the PTM.

Materials and Methods

Rearing technique: The potato tubermoth, (PTM) were reared on potato tubers in the Laboratory of Entomology, National Center for Radiation Research and Technology. Potato tubers were cleaned from dust and parasites by washing and drying with clean towels or tissue papers. A thin layer of clean sand (exposed to high temperature in oven to kill other insects or parasites) was distributed on the bottom of rearing cages to allow the pupation (Hemeide, 1976). The exact duration of (PTM) is from 21 to 25 days under laboratory conditions (27± 2°C and 60± 5 RH).

Preparation of plant extracts: Neem and colocasia fresh green leaves were collected, cleaned and dried at room temperature till dryness. The dried leaves were minced well by using electric machine aqueous extracts were prepared by adding 400g of plant powder to 100ml of distilled water to prepare 4.0% water extract and the other lower concentrations (2.0, 1.0, 0.5 and 0.25%) were prepared by dilution of the stock solution with distilled water.

Field experiments: Different concentrations of both neem and colocasia and their mixtures by ratio of (1:1) were sprayed on the potato plant leaves, then hundred of the newly hatched larvae (neonate) were offered for each plant. After harvest the potato tubers were transferred to the laboratory to investigate the infestation, survived larvae, pupation, emergence and sex ratio percentages. The average weight of resulted pupae (mg) were calculated.

Oviposition deterrence: Newly emerged adults were sexed and allowed to mate in glass jars of 1 liter capacity at the ratio of 10:10 (female:male) the jars were supplied with two strips of tissue paper one of them painted in the aqueous extract of neem or colocasia and the other were painted with distilled water and used as control, the two strips were left till dryness and introduced to the jars. The number of eggs deposited on treated or untreated strips were counted and the obtained results were expressed in terms of oviposition deterrent indices (ODI) as defined by Lundgren (1975) to provide a convenient indication of activity on a scale of zero to 100.

$$ODI = \frac{B-A}{B+A} \times 100$$

Where, A and B are the number of eggs laid on treated and untreated strips, respectively to investigate the combined effect of gamma radiation and plant extracts (neem and colocasia), newly emerged adult females were irradiated with 50 and 100Gy and introduced to cages containing treated and untreated strips of different concentration (4.0, 2.0, 1.0, 0.5 and 0.25%) of neem, colocasia or their mixtures. Percent fecundity and fertility were calculated and oviposition deterrent indices (ODI) were also calculated. Sterility percentage was calculated according to Chamberlain's formula (Guirguis, 1979) .

Irradiation technique: Gamma Cell Unit 220 Located at the National Center for Radiation Research and Technology, Atomic Energy Authority; Nasr City, Cairo, Egypt, was the irradiation source used in present study with a dose rate 4.16 rad/sec.

Statistical analysis: The obtained data were statistically analyzed and the values of LSD. were determined, whenever, the calculated "F" values were significant at 5% and 1% level (Snedecor and Cochran, 1980).

Results and Discussion

Infestation, survived larvae and pupation percentages, average of pupal weight, percentages of emergence and sex ratio were determined (Table 1). Treatments of potato plant with different concentrations of neem, colocasia, or their combination (neem + colocasia) under field conditions decreased significantly the percent infestation of potato tubers compared with untreated ones. Percent infestation decreased significantly with the increase in concentrations from 0.25 to 4.0%. The highest decrease in infestation percentage was noticed in treatment with the mixture of neem and colocasia followed by treatment with neem and finally with treatment by colocasia. The highest concentration (4.0%) prevent the infestation in case of treatment with neem only or with it's mixture with colocasia compared with 88.58% infestation in control.

The number of survived larvae were also decreased significantly by increasing the concentrations but mixture of neem and colocasia at concentration 2.0 % killed all the larvae compared with 11.67, 25.33 and 77.67 in case of treatment with neem, colocasia and the control, respectively. Percent pupation and average pupal weight also decreased with the increase in concentration and the best decrease was observed in case of treatment with mixtures of neem and colocasia. Emergence percentage also decreased in all treatments and the concentration 1.0 prevented all adult emergence in mixture of neem and colocasia. The number of emerged males exceeded that of females in all treatments and in control except at 0.25% in case of treatment with colocasia, the number of males and females are equal (Table 1). The results agree with those of Babu *et al.* (1999) who stated that cowpea protected with aqueous extracts of *Coleus aramaticus, Morinda tinctoria* and *Cassia stamea* were effective in reducing egg laying, adult emergence and percent reduction in weight loss.

Gulati (1998) stated that the number of eggs oviposited was reduced from 147.6 in untreated wheat to 10.2, 21.4 and 27.6 following treatment with 0.4% neem oil, neem ark and neem cake, respectively. Rajapakse *et al.* (1998) found that *Azadirachta indica* gave the highest reduction in oviposition of *Callosobruchus maculatus* followed by *Annona reticulate, Azadirachta indica* caused a significant reduction in adult emergence. Deka *et al.* (1998) used eight aqueous plant extracts *(Azadirachta indica, Clerodendron inerme, Pongamia pinnata, Melia azedarach, Polygonum orientale, Lantana camara, Adhatoda vasica and Cassia tora)* and mentioned that at all the concentrations tested (2-10%) exhibited ovicidal action against *Helopeltis theivora.* The ovicidal action was more promising with an increase in the concentration of extracts. Abou-Fahr *et al.* (2001) stated that the aqueous and methanol extracts of callus, fruit and leaves of *Melia azedarach* have repellent activity and significantly decreased the oviposition rate of *Bemisia tabaci.*

The highest reduction in survived larvae, percentage of pupation and adult emergence by treatment with neem may be due to azadirachtin the most biologically active substance from neem which modifies the programs of insects by influencing hormonal systems especially that of ecdysone Schmutterer (1990). The effects of azadirachtin are both dose and time dependent. They prevent both ecdysis and apolysis and can cause death before and during moulting, possibly inducing"permanent" larvae (Mordue and Blackwell, 1993). The use of neem besides colocasia potentiate each other and gave good result than when each of them used alone.

The fecundity, percentage of fertility, the oviposition deterrent indices (ODI) and percentages of sterility of the females exposed to strips treated with different concentrations of neem, colocasia and their mixtures, (4.0, 2.0, 1.0, 0.5 and 0.25%) are given in Table 2. High deterrence was noticed with higher concentrations and neem + colocasia mixture treatment.

Maximum fecundity was obtained with 0.25% of colocasia (101.33 egg/female), as compared with 122.33 eggs/female in control, while minimum fecundity (0.00 egg/female) was scored

Table 1: Effect of aqueous extracts of neem, colocasia and their mixtures on infestation, survived larvae percentage, pupation percentages, average pupal weight, emergence and sex ratio percentages of the potato tubermoth, *Phthorimaea operculella* (Zeller)

Treatments(%)	Biological aspects						
	Infestation	Survived larvae	Pupation	Av. pupal weight (mg)	Emergence (%)	Sex ratio	
						males	females
Neem							
0.25	74.29	61.00	80.87	12.6	83.78	51.61	48.39
0.5	57.14	50.67	86.42	11.82	76.92	53.75	46.25
1.0	34.29	21.33	42.19	9.83	48.15	61.54	38.46
2.0	22.86	11.67	28.57	8.22	20.0	100.00	--
4.0	0.00	0.00	0.00	0.00	0.00	--	--
Colocasia							
0.25	88.57	73.33	90.00	12.89	92.93	50.0	50.00
0.5	77.14	70.0	81.9	12.02	90.12	52.26	47.74
1.0	60.0	41.67	64.8	10.05	77.78	55.56	44.04
2.0	38.24	25.33	38.16	10.00	48.28	64.29	35.71
4.0	20.00	14.00	0.00	0.00	0.00	--	--
Neem-colocasia							
0.25	71.43	59.33	70.22	9.12	9.12	75.76	24.24
0.5	52.94	38.67	52.17	8.43	8.43	78.26	21.74
1.0	27.27	10.33	18.92	7.88	7.88	--	--
2.0	8.57	0.00	0.00	0.00	0.00	--	--
4.0	0.00	0.00	0.00	0.00	0.00	--	--
Control	88.58	77.67	92.27	13.01	13.01	57.35	42.65
L.S.D. 0.05	2.1	13.9	11.4	2.9	2.9	6.4	3.8
0.01	2.8	18.5	15.3	3.9	3.9	8.7	5.2

Table 2: Effect of aqueous extracts of neem, colocasia and their mixtures on fecundity, fertility percentage, ODI and sterility percentage of the potato tubermoth, *P. operculella* (Zeller)

Treatments (%)	Biological aspects			
	Fecundity (Egg/female)	Fertility (%)	ODI	Sterility (%)
Neem				
0.25	81.67	75.1	19.93	48.46
0.5	58.33	49.34	35.45	75.82
1.0	34.67	28.67	55.81	91.65
2.0	18.33	12.33	73.94	98.10
4.0	0.00	0.00	100.00	100.00
Colocasia				
0.25	101.33	95.07	9.39	19.05
0.5	82.67	82.26	19.35	42.85
1.0	73.33	64.55	25.05	60.22
2.0	59.67	45.25	34.43	77.31
4.0	25.33	18.42	65.69	96.07
Neem-colocasia				
0.25	50.67	56.58	41.42	75.90
0.5	38.33	39.13	52.28	87.40
1.0	22.00	19.7	69.51	96.36
2.0	0.00	0.00	100.00	100.00
4.0	0.00	0.00	100.00	100.00
Control	122.33	97.28	0.00	0.00
L.S.D. 0.05	11.9	8.3		
0.01	16.2	11.1		

Table 3: Effect of aqueous extracts of neem, colocasia, and their mixtures combined with 50 and 100Gy of gamma radiation on fecundity, fertility percentage ODI (oviposition deterrent indices) and sterility percentage of the potato tubermoth, *P. operculella* (Zeller).

	Biological aspects							
	50Gy				100Gy			
Treatments (%)	Fecundity egg/female	Fertility	ODI	Sterility %	Fecundity Egg/female	Fertility	ODI	Sterility %
Neem								
0.25	4.33	36.92	47.69	86.56	27.67	28.92	63.11	93.28
0.5	29.67	24.72	60.69	93.84	17.33	13.46	75.18	98.04
1.0	18.34	5.45	73.92	99.16	0.00	0.00	100.00	100.00
2.0	11.00	0.00	83.5	100.00	0.00	0.00	100.00	100.00
4.0	0.00	0.00	100.00	0.00	0.00	0.00	100.00	100.00
Colocasia								
0.25	52.0	51.28	40.34	77.59	36.33	38.53	54.2	88.24
0.5	38.33	40.00	52.28	87.12	25.67	24.68	65.31	94.68
1.0	30.67	13.04	59.91	96.63	19.33	0.00	72.71	100.00
2.0	21.33	0.00	70.30	100.00	0.00	0.00	100.00	100.00
4.0	15.67	0.00	77.29	100.00	0.00	0.00	100.00	100.00
Neem-colocasia								
0.25	27.67	30.12	6.73	92.99	10.33	0.00	100.00	100.00
0.5	18.33	16.36	73.94	97.48	0.00	0.00	100.00	100.00
1.0	0.00	0.00	100.00	100.00	0.00	0.00	100.00	100.00
2.0	0.00	0.00	100.00	100.00	0.00	0.00	100.00	100.00
4.0	0.00	0.00	100.00	100.00	0.00	0.00	100.00	100.00
Control	122.33	97.28	--	0.00	22.33	97.28	--	
L.S.D. 0.05	9.33	9.5			8.2	7.8		
0.01	11.7	11.28			11.0	10.4		

with 4.0% of neem and 2.0 and 4.0% of neem + colocasia. There was a highly significant reduction in fertility, especially with higher concentrations as it scored 0.00, 18.42 and 0.00% with concentration 4.0% of neem, colocasia and the mixture of neem + colocasia, respectively. Control scored 97.28% fertility. The highest ODI (100.0) was registered with 4.0% of neem extract and 2.0 and 4.0% of neem + colocasia, while the 4.0% of colocasia recorded 65.69 ODI. Percentages of sterility were significantly increased as the concentrations of extracts increased and the highest percentage of sterility (100.0%) was obtained with neem (4.0%) followed by neem + colocasia (2.0 and 4.0%) and finally 4.0% colocasia (96.07%) with 4.0% concentration.

Irradiation of full grown pupae of PTM, with 50 and 100Gy caused highly significant reduction in both fecundity and fertility when adults were exposed to strips treated with different concentrations of neem, colocasia and their mixture (Table 3). The dose 100Gy was more effective than 50Gy e.g., fecundity (after exposure to 50Gy) scored 18.34, 11.00 and 0.00 with neem concentrations 1.0, 2.0 and 4.0%, respectively, while it scored (after exposure to 100Gy) 0.00% with 1.0, 2.0 and 4.0% neem concentration. Colocasia treatments scored 30.67, 21.33 and 15.67% fecundity (after 50Gy exposure) but after 100Gy it scored 19.33, 0.00 and 0.00 with 1.0, 2.0 and 4.0% concentrations, respectively. The previous concentrations scored 0.00% fecundity with both 50 and 100Gy exposure when strips were treated with mixture of neem and colocasia extracts. Fertility was drastically reduced with both 50 and 100Gy as it recorded 0.00 with most treatments especially with mixture of neem and colocasia followed by neem only. Dose of 100Gy caused 100.0 ODI in all treatments with mixture of neem and colocasia followed by neem only with

1.0, 2.0 and 4.0% concentrations. Colocasia only treatments caused the lowest ODI e.g., 54.2, 65.31, 72.71, 100.0 and 100.0 with concentrations 0.25, 0.5, 1.0, 2.0 and 4.0%, respectively. Sterility affected sharply with gamma irradiation especially with 100Gy and the mixture of neem and colocasia which scored 100% sterility in all used concentrations.

From these results we notice that mixture of neem and colocasia cause higher effect than that caused by any of them alone and these results agree with that of Abd El-Salam (1993), who studied the efficiency of neem Azal-S and Margosan-O in controlling *Liriomyza trifolii* on *Vicia faba.* He found that the application of both compounds to *V. faba* seedlings suppressed the number of deposited eggs. The ovicidal effect of the tested compounds were 50.0 and 56.1% of eggs failed to hatch after 1.0% application of either neem-Azal-S or Margosan-O, respectively. Kumari and Kumar (1998) found that a mixture of powdered tobacco leaves and neem seed powder caused a high mortality to *C. chinensis* infesting pulse grains. The decline in oviposition at higher dose was attributed to interference of vitellogenesis and severe damage to the egg chambers in the ovaries (Pandey and Khan, 1999). Rizk (1998) stated that sterility increased with the increase in gamma radiation doses. The increase in sterility was more pronounced with adults previously treated as full-grown larvae with LC_{10} *Azadirachta indica* or *Thevetia nerifolia.*

According to Schmutterer and Rembold (1995) azadirachtin is the primary sterilizing agent of neem- based products and inhibits oogenesis and vitellogenesis by inducing a temporal shift in ecdysteroid and juvenile hormone titers. pure azadirachtin is known as an effective sterilant of female insects in several orders. It could be recommended to use neem extract in PTM control in both storage and field conditions especially if other plant extract mixed with it (like colocasia), as plants have no risk to environment or make health hazards to different organisms. Gamma irradiation played an important role to reduce the risk of PTM. attack and it could be considered a good helping agent in pest control.

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