Tropical Agricultural Research and Extension 5(1 & 2) : 2002

Bioactivity of leaf volatiles of *Azadirachta indica* A. Juss. and *Murraya koenigii* Spreng. against *Sitophilus oryzae* L. (Coleoptera: Curculionidae)

P. A. Paranagama*[1], K. H. T. Abeysekera[1], L. Nugaliyadde[2], K. Abeywickrama[3],
1 Department of Chemistry, University of Kelaniya, Kelaniya, 60011, Sri Lanka, Tel. 914486, Fax 914485, Email priyani123@yahoo.com
2. Rice Research and Development Institute, Bathalagoda, Ibbagamuwa, 60500, Sri Lanka.
3.Department of Botany, University of Kelaniya, Kelaniya, 60011, Sri Lanka, Tel. 914480, Fax 911485, Email kris@kln.ac.lk

Accepted 17th September 2002

ABSTRACT

Repellent activity, fumigant and contact toxicities of leaf volatiles of *Azadirachta indica* A. Juss. and *Murraya koengii* Spreng. were evaluated against *Sitophilus oryzae* L. In the dual choice repellency test with *A. indica* leaf volatiles, significantly higher number of insects was repelled at doses above 100 mg, whereas volatiles of *M. koenigii* attracted insects at 25 mg dose and repelled at 300 mg dose. In the fumigant toxicity test 100% mortality was observed at the concentration of 32.5 mg/ml, 3 days after the treatment with 2 volatiles separately. The LC_{50} values for fumigant toxicities were 13.5 and 22.5 mg/ml for *A. indica* and *M. koenigii* volatiles, respectively. In contact toxicity test, 100% mortality was observed immediately after 48 h contact exposure of insects at concentrations of 0.375 and 0.125 mg/cm^2 of *A. indica* and *M. koenigii* respectively. The respective LC_{50} values were 0.12 and 0.08 mg/cm^2 for *A. indica* and *M. koenigii* leaf volatiles.

Key words: *Azadirachta indica, bioactivity, Murraya koenigii, Sitophilus oryzae*

INTRODUCTION

Insects are the main causative agents for grain deterioration and nearly 90% of the total dry matter loss in storage are due to insect pests (Fernando *et al.* 1988). The control of insect pests is largely based on synthetic pesticides. There are no contact pesticides or fumigants which are safer to control pests in stored food (Chapman and Dyte 1976). Excessive use of synthetic pesticides pollute environment with toxic residues, disrupt natural pest control mechanisms and enhance development of pesticide resistant insect strains (Arthur 1994; Swarnasiri and Palipane 1995). Hence there is an increasing demand to develop safe alternatives to replace synthetic pesticides (Tripathi *et al.* 2000).

Sitophilus oryzae L. (rice weevil) is one of the major pests of stored cereals and the predominant pest of milled rice (Chapman and Dyte 1976). In a previous study fresh leaves of *Azadirachta indica* A. Juss. (Neem) when mixed with paddy at 1% (w/w) effectively controlled *S. oryzae, Rhyzopertha dominica* and *Sitotroga cerealella* (Swarnasiri and Palipane 1995). In India, dried leaves of *A. indica* are mixed with stored grain to protect them from pest damages (Jotwani and Sircar 1965; Ahmed and Grainge 1986). *Murraya koenigii* Spreng. (Curry leaves) leaf steam distillates are found to be effective against *Aphis craccivora* and *Callasobruchus maculatus* (Bandara *et al.* 1990; KANP Bandara personal communication). Furthermore, *M. koenigii* leaves are extensively used for culinary purposes and in indigenous medicine.

Though the insecticidal effect of leaves of *A. indica* has been thoroughly studied on *S. oryzae*, that of the separated volatile fraction has not been studied. Also, there are few studies done on the effect of volatiles of *M. koenigii* on *S. oryzae*. This study was undertaken to evaluate the bioefficacy of the volatiles of leaves of *A. indica* and *M. koenigii* on *S. oryzae* with the view of developing environmentally safer and effective compounds for the control of this pest.

MATERIALS & METHODS

Insects

Adults of *S. oryzae* were obtained from laboratory cultures maintained at 28 ± 3 ^{0}C and 70 80% r. h. in insect room at the University of Kelaniya. Freshly milled white raw rice prepared according to Heinrichs *et al.* (1985) was used as the culture medium. One-week-old adults were used for the bioassays.

Extraction of volatiles

Leaves of *A. indica* and *M. koenigii* were air dried

* Corresponding author

under shade for 24 hours and steam distilled separately (Paranagama 1991). The volatiles were extracted into dichloromethane and the solvent was removed using a rotary evaporator at 40 ^{0}C. The concentrate was dried on anhydrous Na_2SO_4. Remaining traces of dichloromethane were evaporated under a dry N_2 stream. The volatiles were stored at 5 ^{0}C in a refrigerator. Dilutions were made using ethanol (EtOH) for all experiments.

Repellent activity

A "Y" shaped olfactometer was made by connecting three glass tubes (10 cm long, 1 cm diameter) and there was an opening on the intersection of 3 arms for the vacuum pump, which facilitated air circulation in the olfactometer (Bandara 1997). Two tubes of the olfactometer were connected with perforated, plastic, transparent, wide mouthed bottles (250 ml) through lids and the other was used to introduce insects (Fig. 1). Two Whatman no. 1 filter papers (2.5 cm x 2.5 cm), one treated with a known amount of volatiles and the other with equal amount of EtOH, were hung separately in the middle

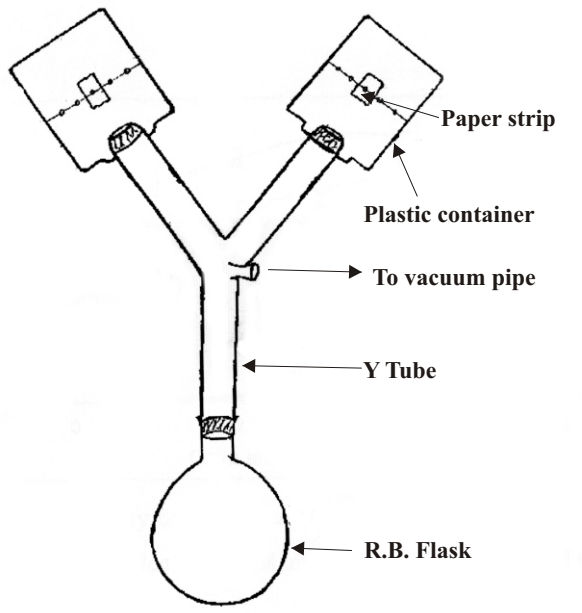

Fig. 1. The Olfactometer

of bottles using metal wires. The olfactometer was placed horizontally on a white background. After switching on the vacuum pump, ten test insects were introduced into the olfactometer. The number of insects moved into the volatile treated and EtOH treated bottles within 30 minutes were recorded. Five doses of *A. indica* (10, 25, 75, 100 & 150 mg) and *M. koenigii* (25, 50, 100, 200 & 300 mg) were tested separately and each dose was replicated 5 times. Placement of volatile treated and EtOH treated filter papers was interchanged randomly in subsequent replicates. This assay was carried out between 07.00 and 10.00 hr of the day. The olfactometer was cleaned using a detergent and dried in an oven after each trial. The means of the number of insects responded to the two treatments at each volatile dose were compared using Chi Square test.

Fumigant toxicity

A Whatnam no. 1 filter paper discs (1 cm diameter), each impregnated with volatiles dissolved in EtOH to give maximum possible concentrations of 10.0, 22.5, 32.5 mg/ml air, *A. indica* and 20, 25, 32.5 mg/ml air, *M. koenigii* were used separately. Each disc was placed on the underside of the screw caps of glass vials (7 ml) separately and the solvent was allowed to evaporate for 10 minutes. The neck of each vial containing 10 insects was blocked with pieces of metal mesh (1 cm diameter). Then the caps were screwed tightly and incubated at 28 ± 3 ^{0}C for 48 h in dark. The same procedure was carried out with similar amount of EtOH and untreated samples were used as the control. Each treatment and control was replicated 5 times. At the end of 48 h fumigant exposure period, the insects were transferred into clean vials containing 3 g of culture medium and mortality was observed for 6 days. The means of mortality of each volatile concentration were compared using Analysis of Variance and Tukey's pair wise comparison test. The LC_{50} values were determined by Probit Analysis computer package, after correcting the mortality values using Abott's formula, when required.

Contact toxicity

Glass vials similar to those used in fumigant toxicity test were used (0.002 m^2 area of the inner surface). Different doses of the test volatiles dissolved in EtOH were applied on the inner surface of vials, to give the final volatile concentrations of 0.125, 0.25, and 0.375 mg/cm^2 of *A. indica* and 0.025, 0.05, and 0.125 mg/cm^2 *M. koenigii*. EtOH was evaporated under dry N_2 stream and 10 insects were introduced into the vial. The rest of the bioassay and conditions were similar to the fumigant toxicity assay.

RESULTS AND DISCUSSION

Repellent activity

When *S. oryzae* was given a choice between the bottles treated with *A. indica* leaf volatiles and EtOH treated bottles, insects were not able to recognize the

volatiles treated bottle at doses lower than 75 mg (Table 1). However, at doses higher than 100 mg, a significant number of insects moved away from the volatile treated bottle. These results indicated that *A. indica* acts as a repellent to these insects at high doses. Insect repellent property of *A. indica* leaves is well documented against various pests (Jotwani and Sircar 1981; Koul *et al.* 1990; Swarnasiri and Palipane 1995). An olfactometer study carried out

Table 1. Percentage of *S. oryzae* moved towards the volatile treated and non-treated bottles during a dual choice repellency test

Volatile	dose	Percentage responded ± S.E.		Σ^2_1	P
	(mg)	Volatile treatment	EtOH Treatment		Value*
A. indica	10	36 ± 7	36 ± 3	0	<0.05
	25	38 ± 7	36 ± 7	0.05	<0.05
	75	18 ± 4	64 ± 5	0	<0.05
	100	18 ± 4	64 ± 5	25.8**	>0.05
	150	16 ± 7	64 ± 2	28.8**	>0.05
M. koenigii	25	50 ± 1.5	24 ± 1	9.1**	>0.05
	50	40 ± 4	44 ± 7	0.2	<0.05
	100	40 ± 11	42 ± 7	0.05	<0.05
	200	36 ± 8	44 ± 11	0.8	<0.05
	300	26 ± 9	46 ± 9	5.5**	>0.05

* p < 0.05, 3.84; ** significant at 5%

with the leaf volatiles of *A. indica* against *C. maculatus* has repelled 73% at the dose of 80 mg (Paranagama, *et al.* 2001a). *Azadirachta indica* oil has shown similar activity as Autan®, a synthetic mosquito repellent, for 10 h of application against *Anopheles tesselatus* (Perera *et al.* 1998).

The repellent effect of leaf volatiles of *M. koenigii* has not been studied extensively. At a dose of 25 mg, significantly higher number of *S. oryzae* moved towards *M. koenigii* volatile enriched bottle indicating a possible attractant activity. No significant difference was observed in insect movement towards the 2 bottles between doses 50 to 200 mg of *M. koenigii* volatiles. Significantly lower number of insects moved away from the volatile treated bottle at the dose of 300 mg. Linalool (0.6%), α-pinene (17.5%) and α-terpeneol (1.6%) are some of the compounds in leaf volatiles of *M. koenigii* (Paranagama 1991) which are also behavior modifying chemicals in many insect orders (Bedoukian 1992). This could be attributed to the possible attractant effect at 25 mg dose of *M. koenigii* volatiles and this effect should be studied further.

Various plant leaves with essential oils have been tested against *S. oryzae* and leaves of *Vitex negundo, Ocimum sanctum, Eucalyptus terreticornis, Curcuma longa* and *Citrus* sp. have reduced *S. oryzae* populations effectively for a period of six months (Swarnasiri and Palipane 1995). The essential oil of *Cymbopogon citratus* at 75 mg dose has repelled 78% of *S. oryzae* (Paranagama *et al.* 2001b).

Fumigant and contact toxicity

The percentage mortality of *S. oryzae* in the control and EtOH treatment was not significantly different from each other (P<0.05), indicating that there was no effect of EtOH on the insect mortality. Both volatiles gave significantly higher mortality as fumigants than the control (Figs. 2 and 3). A direct relationship was observed between mortality of insects and volatile concentration. Both test volatiles showed maximum mortality at the concentration of 32.5 mg/ml 3 days after treatment. Similarly both volatiles indicated the properties of contact toxicants to this insect (Figs. 4 and 5). In the contact toxicity experiment, percentage mortality in treatments was significantly higher than that of the control (P>0.05). The mortality increased with increasing concentration of two test volatiles. Maximum

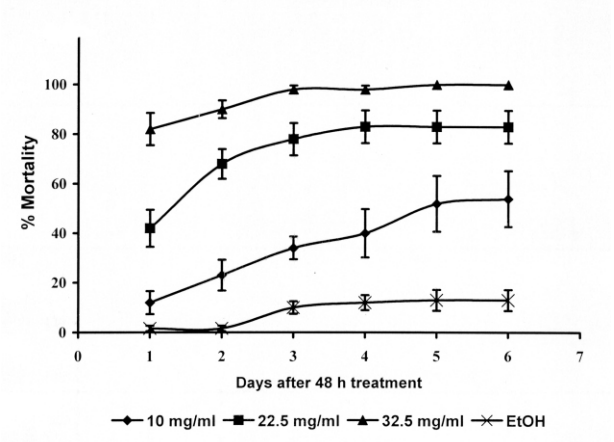

Fig. 2. Mortality of *S. oryzae* after 48 hours of fumigant exposure to different doses of *A. indica* leaf volatiles and fed on rice for a period for 6 days ± S. E.

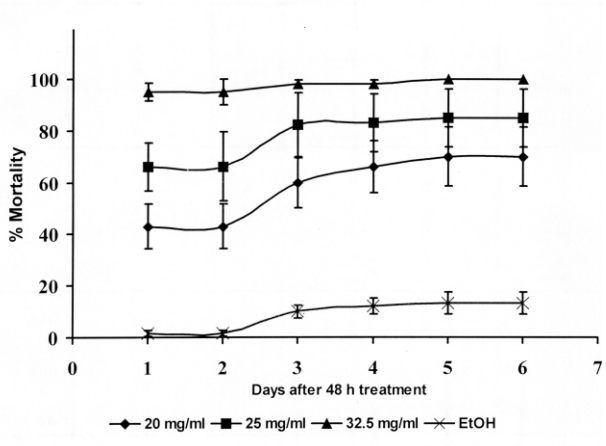

Figure 3. Mortality of *S. oryzae* after 48 hours of fumigant exposure to different doses of *M. koenigii* leaf volatiles and fed on rice for a period for 6 days ± S. E.

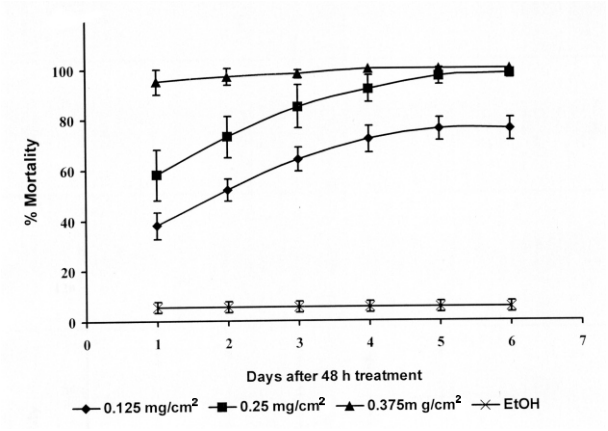

Figure 4. Mortality of *S. oryzae* after 48 hours of contact exposure to different doses of *A. indica* leaf volatiles and fed on rice for a period for 6 days ± S. E.

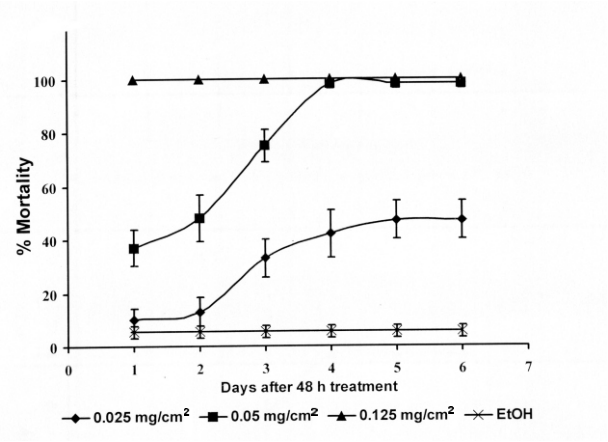

Figure 5. Mortality of *S. oryzae* after 48 hours of contact exposure to different doses of *M. koenigii* leaf volatiles and fed on rice for a period for 6 days ± S. E.

mortality was at 0.375 mg/cm^2 for *A. indica* and 0.125 mg/cm^2 for *M. koenigii* on 24 h after treatment. LC_{50} values for fumigant and contact toxicities of *A. indica* and *M. koenigii* revealed that contact toxicities of both volatiles were more effective than the fumigant toxicity (Table 2).

The volatiles extracted from dried leaves of *A. indica* had previously been pesticidal on paddy pests, and macerated leaf juice has been reported to be larvicidal on weevils (Gunasena and Marambe 1998). Paranagama *et al.* (2001a) reported that the LC_{50} values fumigant and contact toxicities of *A. indica* were 0.35 g/l and 1.07 g/m^2 as respectively against *C. maculatus*.

The insecticidal effect *M. koenigii* has not been extensively studied. The steam distillate of *M. koenigii* had shown 45% mortality at 24 h after treatment against *A. craccivora* (Bandara *et al.* 1990). Volatile essential oil of dried leaves of *M. koenigii* has shown 100% mortality and reduction of F_1 emergence of *Callasobruchus chinensis* at 340 ppm when used as a fumigant and as a contact

Table 2. LC_{50} values of leaf volatiles of *A. indica* and *M. koenigii* to *S. oryzae*

Volatile	LC_{50} Value*	
	Fumigant (mg/ml)	Contact (mg/cm^2)
A. indica	13.5	0.12
M. koenigii	22.5	0.08

* 24 hours after treatment

toxicant (Pathak *et al.* 1997).

The essential oil of *C. citratus* has shown fumigant and contact toxicity effect on *S. oryzae.* The results revealed that LC_{50} values were 1.14 mg/ml and 0.78 mg/cm^2 respectively (Paranagama *et al*. 2001b). Toxicity of several compounds and plant oils has been extensively studied against *S. zeamais*. Essential oil of *Ocimum kilimandscharicum* has shown 100% mortality on insects at 0.3 g oil / 250 g maize (Jembera *et al.* 1995). Cinnamaldehyde has shown fumigant and contact LC_{50} values of 0.54 mg/cm^3 and 0.7 mg/cm^2 respectively (Huang and Ho 1998), whereas LC_{50} values of nutmeg oil were 7.7 mg/cm^3 and 1.7 mg/cm^2 for fumigant and contact toxicities (Huang *et. al.* 1997).

CONCLUSION

The present study on biological activity of *A. indica* and *M. koenigii* volatiles revealed that these two plants had the potential to be developed as insect pest controlling agents. Volatiles of *M. koenigii* showed the highest potential as a contact toxicant whereas volatiles of *A. indica* were found to be a potent fumigant and a repellent against *S. oryzae*. The organoleptic and safety aspects of these two volatiles are yet to be evaluated.

ACKNOWLEDGEMENTS

Authors gratefully acknowledge the financial assistance provided by National Science Foundation and National Research Council, Sri Lanka. The assistance of Dr. U. Chandrasekera of University of Kelaniya in statistical analysis and the technical staff of Departments of Chemistry, Botany and Rice Research and Development Institute are also acknowledged.

REFERENCES

Ahmed S and Grainge M 1986 Potential of the Neem tree (*Azadirachta indica*) for pest control and rural development. Economic Botany. 40(2): 201-209.

Arthur FH 1994 Grain protectant chemicals: present status and future trends. *In*: Highley E, Wright

EJ, Banks HJ and Champ BR (ed.) Proceedings of the 6th International Working Conference on Stored Product Protection, Canberra, Australia, pp. 719-721.

Bandara BMR, Hewage CM, Jayamanne DHIW, Adikaram NKB, Bandara KANP and Pinto MRM 1990 Biological activity of some steam distillates from leaves of ten species of Rutaceous plants. Journal of National Science Council of Sri Lanka. 18(1): 71-77.

Bandara KANP 1997 Insecticidal components from some Sri Lankan plants. Ph. D. Thesis, University of Peradeniya, Sri Lanka. p. 176.

Bedoukian RH 1992 Regulation of some class of phytochemicals: flavor and fragrance ingredients related to insect behavior-modifying chemicals. In: Ridgway RL, Inscoe M, Arn H (ed.) Insect pheromone and other behavior modifying chemicals Monograph No. 51, British Crop Protection Council, Surrey, U. K, pp. 79-82.

Chapman BR and Dyte CE 1976 Report of the FAO global survey of pesticide susceptibility of stored grain pests. FAO Plant Production and Protection Series no.5, FAO of the United Nations, Rome.

Fernando HD, Palipane KB and Adhikarinayake TB 1988 Improvements on farm level grain storage methods in Sri Lanka. Sri Lankan Journal of Post harvest Technology. 1 (1): 47-67.

Gunasena HMP and Marambe B 1988 Neem in Sri Lanka. Monograph, University of Peradeniya, Sri Lanka, pp. 37-53.

Heinrichs EA, Medrano FG and Rapusas HR 1985 Genetic evaluation for insect resistance in rice. International Rice Research Institute, Manila, Philippines, pp. 307-317.

Huang Y and Ho SH 1998 Toxicity and antifeedent activities of cinnamaldehyde against the grain storage insects, *Tribolium castaneum* (Herbst.) and *Sitophilus zeamais* Motsch. Journal of Stored Product Research. 34(1): 11-17.

Huang Y, Tan JMWL, Kini RM and Ho H 1997 Toxic and antifeedent action of nutmeg oil against *Tribolium castaneum* (Herbst.) and *Sitophilus zeamais* Motsch. Journal of Stored Product Research. 33(4): 289-298.

Jembera B, Obeng Ofori D, Hassanali A and Nyamasyo GNN 1995 Products derived from the leaves of *Ocimum kilimandscharicum* (Labiatae) as post-harvest grain protectants against infestation of three major stored product insect pests. Bulletin of Entomological Research. 85(3): 361-367.

Jotwani MG, Sircar P 1965 Neem seed as a protectant against stored grain pests infesting wheat seeds. Indian Journal of Entomology, **27**: 160-164.

Koul O, Isman MB and Ketkar CM 1990 Properties and uses of neem, *Azadirachta indica*. Canadian Journal of Botany. 68: 1-11.

Paranagama PA 1991 Analysis of Sri Lankan essential oils by Gas Chromatography and Mass spectroscopy. Senanayake, U. M. (ed.) Industrial Technology Institute, Colombo, pp. 1 - 36.

Paranagama PA, Adhikari AACK, Bandara KANP and Abeywickrama KP 2001a Insecticidal and repellent activity of volatile leaf constituents of *Azadirachta* indica A.Juss. (neem) against *Callasobruchus macultus* (L.) inhabiting stored cowpea Proceedings of the 57th Annual Session, Sri Lanka Association for the Advancement of Science, p. 162.

Paranagama PA, Jayaratne KHT, Nugaliyadde L and Abeywickrama KP 2001b Bioactivity of the essential oil of *Cymbopogon citratus* (lemongrass) on *Sitophilus oryzae* L. (Coleoptera: Curculionidae). Proceedings of the 57th Annual Session, Sri Lanka Association for the Advancement of Science, p.161.

Pathak N, Yadav T D, Jha AN and Vasudevan P 1997 Contact and fumigant action of volatile essential oils of *Murraya koenigii* against *Callasobruchus chinensis*. Indian Journal of Entomology. 59(2): 198-202.

Perera KLRL, Rajakaruna J, Wickremasinghe R and Wickremasinghe AR 1998 Mosquito repellent activity of citronella and neem oil. Proceedings of the 547th Annual Session, Sri Lanka Association for the Advancement of Science, pp. 17 - 18.

Swarnasiri DPC and Palipane KB 1995 Use of plant material for insect control in farm level grain storage. RPRDC Technical report no. 33/95. Rice Processing, Research and Development Centre of Paddy Marketing Board, Anuradhapura, Sri Lanka, p. 8.

Tripathi AK, Prajapati V, Aggrawal KK, Khanuja SPS and Kumar S 2000 Repellency and toxicity of oil from *Artimisia annua* to certain stored product beetles. Journal of Economic Entomology. 93(1): 43-47.

Wickramananda IR, Mordue (Luntz) AJ and Allan EJ 1998 Insecticidal activity of leaf extracts of Neem (*Azadirachta indica* A. Juss.). Tropical Agricultural Research. 10: 282-290.