



ISSN: 0974 - 0376

The Ecoscan : Special issue, Vol. VII: 221-226: 2015
AN INTERNATIONAL QUARTERLY JOURNAL OF ENVIRONMENTAL SCIENCES
www.theecoscan.in

IMPACT OF SOME PLANT PRODUCTS AS GRAIN PROTECTANTS AGAINST LESSER GRAIN BORER, *RHIZOPERTHA DOMINICA* FAB. IN STORED SORGHUM

Abhimnyu Singh *et al.*,

KEYWORDS

Rhizopertha dominica
Sorghum
Plant products
Germination

Proceedings of National Conference on
Harmony with Nature in Context of
Bioresources and Environmental Health
(HARMONY - 2015)
November 23 - 25, 2015, Aurangabad,
organized by
Department of Zoology,
Dr. Babasaheb Ambedkar Marathwada University
Aurangabad (Maharashtra) 431 004
in association with
NATIONAL ENVIRONMENTALISTS ASSOCIATION, INDIA
www.neaindia.org



ABHIMNYU SINGH*, ANUJ SINGH, JHUMAR LAL AND K. C. SHARMA

Department of Entomology, Rajasthan College of Agriculture,
Maharana Pratap University of Agriculture and Technology, Udaipur (Raj) -313 001, INDIA
e-mail: abhimanyu.abhi031@gmail.com

ABSTRACT

A laboratory experiment was conducted to assess the impact of three plant products namely custard apple seed powder (*Annona squamosa* L.), neem seed kernel powder (*Azadiracta indica* A. Juss) and Jamun seed powder (*Syzygium cumini* L.) against *Rhizopertha dominica* in stored sorghum in 2014. All plant products were found effective against lesser grain borer as compared to control. The sorghum grains treated by neem seed kernel powder at 10g/kg grains was found to be superior with minimum grain damage of 0.30 per cent, weight loss 0.10 per cent, adults emergence 0.17 and 99.00 per cent adult mortality. While least effective treatments was Jamun seed powder at 5g/kg grains with 70.00 per cent adult mortality, 8.67 adults emergence, 8.16 per cent grain damage and 6.27 per cent weight loss. No adverse effect of inert dusts and plant products has been observed on the germination of sorghum grains upto 120 days after storage.

INTRODUCTION

Sorghum, *Sorghum bicolor* (L.) Moench is one of the important food, feed and fodder crop of our country. It is the gifted genus of the tropical regions that provide food, feed, fodder and fuel to millions of poor farmer families and their livestock's. It is one of the most important crops in the world because of its adaptation to a wide range of ecological conditions, suitability for low input cultivation and diverse uses (Doggett, 1988). It is a staple food crop for millions of the poorest and most food-insecure people in the semi-arid tropics of Africa, Asia and Central America. Worldwide it is cultivated on 40 million ha with a production of 60 million tones. In India, it covers about 6.32 m ha with an annual grain production of 6.03 million tones and productivity of about 954 kg/ha (Anonymous 2013).

Farmers retain about 60-70 per cent of their produce for seed purpose, home consumption and for sale (Reddy and Pushpamma., 1980). Though, food grain production of 265 million tonnes was achieved during 2013-14, it is believed that nearly 15 per cent of the grains stored after each harvest will be lost due to ravages by rats, insects, non insect pests, microbial agents and spillage (Walter, 1971). Among these, insects cause loss to the tune of 6.58 per cent. A survey conducted by Mookerjee *et al.* (1998) revealed that the annual loss of grains due to insects is estimated to 5.90 million tones, reflecting the intensity of insect pest problem in store. The presence of insects in stored products has always posed unique problems. The average loss of food grains in storage due to biotic and abiotic factors accounts for 10 per cent per year, out of which insects are contributing about 2.5 to 5.0 per cent. It is reported that major loss of food grains in storage is contributed by internal feeders viz., lesser grain borer, *Rhizopertha dominica* Fab. Survey conducted by FAO revealed that, *R. dominica* is the major pest of wheat, rice and millets in India (Champ and Dyte, 1977). The lesser grain borer, *R. dominica* was recorded as a major pest from all district of Rajasthan (Bhargava and Choudhary, 2007).

The present, pest control measures in storage rely on the use of synthetic insecticides and fumigants. Their indiscriminate use in the storage, however, has led to a number of problems including insect resistance, toxic residues in food grains (Fishwick, 1988), environment pollution (WMO, 1995) and increasing cost of application. Currently only two fumigants, methyl bromide and phosphine are widely used against stored product insect pests. According to the 1997 decision of the 9th Montreal Protocol, methyl bromide, which is a proven ozone depletor in the atmosphere, will be phased out by developed countries and by 2015 in the developing countries. To avert these problems, there is a need to develop management practices, which are more effective, economical safer and easier to adopt. The use of plant products are considered to be the novel approaches, though they are good age old methods, ecologically safe, easily degradable, besides easy availability at low cost. Plant material based insecticides are target specific, non-toxic to human being and beneficial organisms, less prone to insect resistance and resurgence, biodegradable and less expensive and are promising grain protectants. Plant materials constitute a rich source of bio-active chemicals (Wink, 1993), hence they could lead to the development of new classes of safer insect control agents. There are encouraging

*Corresponding author

reports on the use of certain indigenous plant products as grain protectants (Sundria *et al.*, 2001 and Bhargava and Meena, 2002). Keeping in view, the present study was conducted with the eco-safe management of lesser grain borer through plant products in stored sorghum.

MATERIALS AND METHODS

Rearing and maintenance of culture

The experiment was conducted in laboratory condition. *R. dominica* adults used in the experiments were collected from RCA Farm, Udaipur in India and kept in the laboratory for mass culture on sorghum at $27 \pm 2^\circ\text{C}$ and $65 \pm 5\%$ RH. The populations were initially collected from sorghum in different storage conditions (bulk and bag storage etc.) The stock culture of the lesser grain borer was reared in laboratory in glass jars of 2 kg capacity containing the sorghum grains. The cultures was maintained for use various investigations undertaken.

Plant products

Plant materials *viz.*, custard apple seed powder (*Annona squamosa* L.), neem seed kernel powder (*Azadiracta indica* A. Juss) and Jamun seed powder (*Syzygium cumini* L.) were collected and shade dried until they became crisp dry. The powder of these materials was obtained by grinding their dried form separately in a mechanical grinder and by sieving through a mesh size of 50. The resulting powder was used directly admixture to the grains.

Experimentation

Different treatment of plant products *viz.*, custard apple seed powder, neem seed kernel powder and Jamun seed powder were evaluated by treating 100 g of sorghum grains in each jar with two application rates of 0.5 and 1.0 per cent of grain weight against *R. dominica* upto 120 days. Each treatment was replicated three times. Untreated check was also used for comparison. Ten pairs of lesser grain borer adult were released in each jar. The mouth of jars were covered with muslin cloth and secured by rim of lid to disallow the entry or exit of test insect. Each plastic jars were examined periodically at monthly intervals to study the, per cent weight loss, per cent grain damage and progeny development (no. of adults).

Adult mortality (%)

The numbers of dead insects in each jar were counted upto 120 days at an interval of 30, 60, 90 and 120 days.

Progeny development (no. of adults)

The observation on population build up was recorded by sieving the grains and counting the total number of adult beetles at monthly intervals.

Grain damage (%)

Before estimating the damage grains, the grains were thoroughly mixed and samples of 100 grains were drawn for five times from each bottle, then damage grains in 100 seeds were counted for each sample.

Weight loss (%)

The observation on per cent weight loss was recorded by counting number of un-infested grains and number of infested

grains. Weight loss was worked out by using the following formula (Adams and Schulton, 1978).

$$\text{Per cent weight loss} = \frac{(\text{UND}) - (\text{DNU})}{\text{U}(\text{ND} + \text{NU})} \times 100$$

Where

U = Weight of uninfested seeds (g)

NU = Number of uninfested seeds

D = Weight of infested seeds (g)

ND = Number of infested seeds

Germination (%)

The effect of inert dusts was studied on germination of sorghum grains at 0, 30, 60, 90 and 120 days of treatment. The germination test was carried out according to International Rules of Seed Testing (Anonymous, 1976).

Statistical analysis

The data recorded were subjected to statistical analysis as Completely Randomized Design (CRD). The data in percentages were transformed in to angular and number values were transformed to square root transformations before statistical analysis.

RESULTS AND DISCUSSION

Adult mortality (%)

The mortality of *R. dominica* in different plant products ranged from 70.00% to 99.00 per cent upto 120 days of storage (Table 1). The significantly higher mortality of 99.00 per cent was recorded in neem seed kernel powder at 10g/kg grains. It was followed by custard apple seed powder at 10g/kg grains with 97.00 per cent. The minimum adult mortality of 70.00 per cent was recorded in jamun seed powder at 5g/kg grains. These findings are in agreement with the findings of Mahanti (2002) reported that neem seed powder at the rate of 2.0 g/kg of maize grain caused 100 per cent mortality of *S. Oryzae* at ten days after beetle release.

Progeny development (no. of adults)

The progeny development of *R. dominica* in different plant products ranged from 0.17 to 8.67 adults in different plant products upto 120 days of storage (Table-1). Significantly higher progeny development with 8.67 adults was recorded in jamun seed powder at 5g/kg grains. The minimum progeny development with 0.17 adults was recorded in neem seed kernel powder at 10g/kg grains. Kumawat (2009) found that plant products neem leaf, neem seed kernel, karanj leaf, dharek leaf and custard apple seed powder at three dose levels, *viz.*, 1.0, 2.5 and 5.0 g/100 g grain were found effective against lesser grain borer, *R. dominica*. There was no adult emergence, grain damage and weight loss up to 90 and 270 days of storage. Latha and Naganagoud (2015) observed number of adults emerged per 100g of sorghum seeds after 120 days of treatment was minimum (59.17 adults/100 g of seeds) in seeds treated with sweet flag rhizome powder with talc as a carrier at 2% concentration.

Grain damage (%)

The grain damage due to *R. dominica* in different plant

Table 1: Effect of different plant products on mortality and progeny development of lesser grain borer.

S. No. Plant product	Dose (g/kg grains)	Mortality(%) at			Progeny development(No. of adults) at						
		30DAS	60DAS	90DAS	120DAS	Mean	30DAS	60DAS	90DAS	120DAS	Mean
1. Custard apple seed powder	5	95.67(77.99)*	96.00(78.52)	91.33(73.28)	90.33(72.21)	93.33(75.50)	0.33(0.91)**	1.00(1.22)	3.33(1.96)	5.33(2.41)	2.50(1.63)
2. Custard apple seed powder	10	100.00(90.00)	100.00(90.00)	95.00(77.08)	93.00(74.66)	97.00(82.94)	0.00(0.71)	0.00(0.71)	1.67(1.47)	2.67(1.78)	1.09(1.17)
3. Neem seed kernel powder	5	94.00(76.32)	95.33(78.34)	91.33(73.13)	89.67(71.44)	92.58(74.81)	0.67(1.08)	1.67(1.47)	3.67(2.04)	6.00(2.55)	3.00(1.79)
4. Neem seed kernel powder	10	100.00(90.00)	100.00(90.00)	100.00(90.00)	96.00(78.46)	99.00(87.12)	0.00(0.71)	0.00(0.71)	0.00(0.71)	0.67(1.08)	0.17(0.80)
5. Jamun seed powder	5	65.00(53.73)	70.67(57.21)	74.00(59.35)	70.33(57.00)	70.00(56.86)	3.67(2.04)	5.33(2.41)	9.00(3.08)	16.67(4.14)	8.67(2.92)
6. Jamun seed powder	10	80.00(63.48)	85.67(67.36)	88.33(70.17)	83.00(65.72)	84.25(66.68)	2.33(1.68)	3.00(1.87)	5.67(2.48)	10.67(3.34)	5.42(2.34)
7. Control		0.33(3.29)	2.67(9.40)	6.33(14.57)	14.33(22.24)	5.92(12.38)	28.33(5.37)	45.00(6.74)	63.67(8.01)	78.00(8.86)	53.75(7.25)
SEm ±		1.18	1.43	1.54	1.39	1.39	0.03	0.03	0.04	0.05	0.04
CD (p = 0.05)		5.58	4.34	4.69	4.23	4.71	0.08	0.10	0.13	0.15	0.12

* Figures in the parentheses are retransformed values, DAS- Days after storage; ** Figures in the parentheses are square root retransformed values

Table 2: Effect of different plant products on grain damage and weight loss caused by lesser grain borer

S.No. Plant product	Dose(g/ kg grains)	Grain damage(%) at			Weight loss(%) at						
		30DAS	60DAS	90DAS	120DAS	Mean	30DAS	60DAS	90DAS	120DAS	Mean
1. Custard apple seed powder	5	1.21(6.31)*	1.34(6.65)	1.39(6.77)	2.41(8.93)	1.59(7.17)	0.46(3.89)*	0.52(4.13)	0.95(5.59)	1.23(6.37)	0.79(5.00)
2. Custard apple seed powder	10	0.00(0.00)	0.00(0.00)	0.40(3.63)	0.83(5.23)	0.31(2.22)	0.00(0.00)	0.00(0.00)	0.36(3.44)	0.74(4.93)	0.28(2.09)
3. Neem seed kernel powder	5	1.30(6.55)	2.00(8.13)	3.42(10.66)	6.54(14.81)	3.32(10.04)	0.50(4.05)	0.74(4.93)	1.12(6.07)	1.30(6.55)	0.92(5.40)
4. Neem seed kernel powder	10	0.00(0.00)	0.00(0.00)	0.00(0.00)	1.20(6.29)	0.30(1.57)	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.40(3.63)	0.10(0.91)
5. Jamun seed powder	5	3.76(11.18)	6.36(14.61)	9.36(17.81)	13.16(21.27)	8.16(16.22)	3.76(11.18)	4.62(12.41)	6.34(14.58)	10.37(18.78)	6.27(14.24)
6. Jamun seed powder	10	2.88(9.77)	4.12(11.71)	6.48(14.75)	9.64(18.09)	5.78(13.58)	2.00(8.13)	3.84(11.30)	4.86(12.73)	6.64(14.93)	4.34(11.77)
7. Control		28.00(31.94)	33.00(35.06)	38.67(38.45)	43.67(41.36)	35.84(36.70)	23.67(29.11)	24.63(29.75)	28.55(32.29)	34.70(36.09)	27.89(31.81)
SEm ±		0.19	0.21	0.24	0.28	0.23	0.16	0.17	0.19	0.22	0.19
CD (p = 0.05)		0.57	0.64	0.74	0.84	0.70	0.50	0.53	0.59	0.68	0.57

* Figures in the parentheses are retransformed values, DAS- Days after storage

Table 3: Effect of different plant products on germination of sorghum grains

S. No.	Plant product	Dose (g/kg grains)	Germination (%) at				
			ODAS	30DAS	60DAS	90DAS	120DAS
1.	Custard apple seed powder	5	87.33(69.34)*	86.67(68.62)	84.00(66.45)	80.67(63.94)	76.00(60.68)
2.	Custard apple seed powder	10	87.67(69.44)	86.00(68.20)	86.33(68.52)	84.00(66.56)	79.33(63.04)
3.	Neem seed kernel powder	5	88.33(70.17)	87.00(68.99)	84.33(66.77)	80.67(63.97)	79.33(63.01)
4.	Neem seed kernel powder	10	86.00(68.03)	85.33(67.52)	83.00(65.68)	78.67(62.50)	76.00(60.68)
5.	Jamun seed powder	5	88.67(70.37)	88.67(70.36)	86.67(68.62)	84.67(66.97)	78.33(62.26)
6.	Jamun seed powder	10	87.67(69.58)	87.33(69.26)	86.67(68.70)	84.33(66.77)	80.67(63.98)
7.	Control		86.00(68.14)	85.67(67.87)	85.67(67.87)	77.67(61.81)	76.00(60.68)
SEm +			1.51	1.56	1.58	1.28	1.15
CD (p=0.05)			NS	NS	NS	NS	NS

products ranged from 0.30 to 8.16 per cent upto 120 days of storage (Table 2). Significantly higher grain damage of 8.16 per cent was recorded in jamun seed powder at 5g/kg grains. The minimum grain damage of 0.30 per cent was recorded in neem seed kernel powder at 10g/kg grains. Results gets support from Singh and Singh (2005) who reported that among seven botanicals significantly less grain damage of 4.47 per cent was recorded in neem seed kernel powder 10 g/kg grain due to *R. Dominica* in wheat. Neem seed kernel powder had to be remixed with sorghum grains after 4 months of storage for lowering infestation by insects and (Das and Borah, 1997). Sweet flag rhizome powder with talc as a carrier at 2% concentration reduced the sorghum seed damage to 35.67 per cent as against 98.67 per cent in untreated check after 120 days of treatment (Latha and Naganagoud, 2015).

Weight loss (%)

The maximum weight loss 6.27 per cent was recorded in jamun seed powder at 5g/kg grains (Table-2). The minimum weight loss 0.10 per cent was recorded in neem seed kernel powder at 10g/kg grains. Singh and Singh (2005) also opined that neem seed kernel powder recorded significantly maximum germination of 81.25 per cent and grain weight loss of 2.08 per cent against *R. Dominica* in wheat. Quadri and Rao (1977) observed promising results with neem seed extract in controlling *C. chinensis* and *R. dominica*. Kumar *et al.* (2005) opined that custard apple seed powder at 1.0 per cent was effective in controlling *S. Oryzae* on sorghum grains upto 90 days after storage. The lowest mean grain damage, weight loss and germination loss were recorded in case of 1 pair of adult pulse beetle i.e., 7.79, 1.81 and 4.55 per cent (Jat *et al.*, 2013). Sorghum seeds treated with sweet flag rhizome powder along with talc as carrier at two percent as it recorded 2.50 per cent seed weight loss after 120 days (Latha and Naganagoud, 2015).

Effect of plant products on the germination of sorghum grains

The effect of three plant products (Table-3) viz., custard apple seed powder, neem seed kernel powder, and Jamun seed powder were studied on germination of sorghum grains at two application rates viz., 5g and 10g/kg grains. The results revealed that no adverse effect of custard apple seed powder, neem seed kernel powder and Jamun seed powder on germination of sorghum grains was observed upto 120 days of treatment. No adverse effect on grain viability of green gram by seed and leaf powders of custard apple at 3.0 per cent was as reported as Saxena *et al.*, 1976, Yadav and Bhatnagar, 1987,

Gundannavar and Deshpande, 2006 and Lakshmi and Venugopal, 2007 and on pigeon pea (Sharma *et al.*, 2010) up to 180 days of treatments was observed, corroborate the present findings. No work on the effect of Jamun seed powder on the germination of sorghum grains is available, therefore, the present results could not be compared. No adverse effect of neem seed kernel powder (5 and 10g/kg grain) was observed on grain viability of sorghum grain. These results are conformity with that of Kumawat (2009) who reported that mixing of neem seed kernel, neem leaf, garden mint leaf, holy basil leaf, henna leaf, marigold leaf, karanj leaf, dharek leaf and custard apple seed powder with sorghum grains had no adverse effect on germination up to 270 days. Das and Borah (1997) reported that the neemseed kernel powder had to be remixed with sorghum grains after 4 months of storage for lowering infestation by insects and good germination.

REFERENCES

- Adams, J. M. and Schulten, G. G. M. 1978. Loss caused by insects, mites and microorganisms in Post-Harvest Grain Loss Assessment Methods (Eds. K. L. Harries and C. J. Lindblad), *American Association of cereal Chemists*. pp. 83-94.
- Anonymous 1976. International Rules for Seed Testing. *Seed Science and Technology*. 4: 1.
- Anonymous 2013. Annual Report 2012-2013. Department of Agriculture and Cooperation Ministry of Agriculture Government of India. *Krishi Bhawan*, New Delhi, p. 4.
- Bhargava, M. C. and Choudhary, R. K. 2007. Grain infestation in stored product with relation to storage structures/receptacles used in different parts of Rajasthan. *National Conference on "Organic Waste Utilization and Eco-friendly Technologies for crop Protection"*. *Extended Abstract*. pp. 147-149.
- Bhargava, M. C. and Meena, B. L. 2002. Efficacy of some vegetable oils against pulse beetle, *Callosobruchus chinensis* (Linn.) on cowpea, *Vigna unguiculata* (L.). *Indian J. Plant Protection*. 30: 46-50.
- Champ, B. R. and Dyte, C. E. 1977. Global survey of pesticide susceptibility of stored grain pests. *FAO, Plant Protection Science*, No. 5, FAO, Rome.
- Das, A. and Borah, B. 1997. Comparative efficacy of certain insecticides against *Sitophilus oryzae* (Linn.) and *Sitotroga cerealella* (Oliv.) in stored wheat. *J. the Agricultural Science Society of North East India*; 10(2): 275-280.
- Doggett, H. 1988. Sorghum, Longman Scientific and Technical, Essex, England, UK. p. 512.
- Xin, Z., Wang, M. L., Barkley, N. A., Burow, G., Franks, C., Pederson, G. and Burke, J. 2008. Applying genotyping (TILLING) and phenotyping analyses to elucidate gene function in a chemically

induced sorghum mutant population. *BMC Plant Biology*. **8**: 103.

Fishwick, F. B. 1988. Pesticide residues in grain arising from post harvest treatments. *Aspects of Applied Biology*. **17**: 37-46.

Gundannavar, K. P. and Deshpande, V. K. 2006. Effect of indigenous products on seed quality and incidence of pulse beetle, *Callosobruchus chinensis*, in different varieties of soybean. *Karnataka J. Agricultural Sciences*. **19**: 393-395.

Jat, N. R., Rana, B. S. and Jat, S. K. 2013. Estimation of losses due to pulse beetle in chickpea. *The Bioscan*. **8(3)**: 861-863.

Kumar, S., Naganagoud, A. and Patil, B. V. 2005. Evaluation of botanical powders against rice weevil (*Sitophilus oryzae*) in stored sorghum. *Karnataka J. Agricultural Sciences*. **18(4)**: 1117-1120.

Kumawat, K. C. 2009. Efficacy of plant powders against lesser grain borer, *Rhyzopertha dominica* (Fabricius) in stored wheat, *Triticum aestivum* Linnaeus. *J. Insect Science*. **22**: 448-452.

Latha, H. C. and Naganagoud, A. 2015. Effect of sweet flag rhizome, *Acorus calamus* L. formulations against *Sitophilus oryzae* in sorghum. *The Bioscan*. **10(3)**: 1213-1218.

Laxmi, L. G. and Venugopal, M. S. 2007. Effectiveness of powdered plant products as grain protectants against the pulse beetle, *Callosobruchus maculatus* (F.). *J. Entomological Research*. **31**: 75-78.

Mookerjee, P. B., Jotwani, M., Sircar, P. and Yadav, R. I. 1998. Studies on the incidence and extent of damage due to insect pests of stored seeds. *Indian J. Ent.* **30**: 61-65.

Quadri, S. S. H. and Rao, B. 1977. Effect of Oleoresin in combination with neem seed and garlic extracts against house hold and stored products. *Pests*. **14(3)**: 11-14.

Reddy, U. K. and Pushpamma, P. 1980. Effect of insect infestation and storage on the nutritional quality of different varieties of pigeonpea. *Proceedings of International Workshop on Pigeonpea*, ICRISAT, pp. 451-453.

Saxena, B. P., Koul, O. and Tikku, K. 1976. Non-toxic grain protectants against the stored grain insects. *Bulletin of Grain Technology*. **14**: 190.

Sharma, K. C., Jain, N. K., Mishra, B. K. and Kumar, S. 2010. Bio - efficacy of plant products of custard apple, *Annona squamosa* (L.) against pulse beetle, *Callosobruchus maculatus* L. *Indian J. Applied Entomology*. **24**: 54-57.

Singh, R. K. and Singh, A. K. 2005. Efficacy of different indigenous plant products as grain protectant against *Rhyzopertha dominica* Fab. on wheat. *Indian J. Entomology*. **67**: 196-198.

Sundria, M., Kumar, J. and Kumar, A. 2001. Efficacy of different botanicals against *Callosobruchus chinensis* (Linn.) in stored green gram. *Indian J. Applied Entomology*, **16**: 1-5.

Walter 1971. Sorptive dust for pest control. *Annual Rev. Ent.* **16**: 123-15.

Wink, M. 1993. Production and application of phyto-chemicals from an agricultural perspective. In *Phytochemistry and Agriculture*, vol 34, ed. TAV Beck, H Breteler, Clarendon, Oxford, UK. pp. 171-213.

WMO 1995. Scientific assessment of ozone depletion: World Meteorological Organization Global Ozone Research and Monitoring Project. Report No. 37, WMO, Geneva, Switzerland.

Yadav, S. R. S. and Bhatnagar, K. N. 1987. A preliminary study on the protection of stored cowpea grains against pulse beetle by indigenous plant products. *Pesticides*. **21**: 25-28.