Root growth and Mitotic effect of nemagon and nemacur on *Vicia faba*

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INTRODUCTION

The progress in the methods of chemical control of nematodes has made its way during the past years. However, most of the research works have been conducted to study the effect of nematicides on crop yield and nematode population, (Bindar and Chabra (1972), Smith *et al*. (1973), Ranjit *et al*. (1974), Singh (1974) and Miller and Saul Rich (1974).).

In the present work the effect of two nematicides, nemagon (haloginated aliphatic compound) and nemacur (phosphorus aromatic compound) on root growth and mitosis of *Vicia faba* was studied.

Material and Methods

Vicia faba seeds (Var. Rebaya 40) were subjected to two types of experiments:-

Field experiments:-

In these experiments the actual field doses used in bean fields were applied. For nemagon, 7.5, 6.2 and 5 ppm concentrations were prepared from the original solution and for nemacur 3.8, 2.8 and 1.9 ppm concentrations were prepared from the original solid.

Seeds were incubated in water for 24 hours then sown in pots containing 5 kgm. sow dust. The sow dust was mixed thmroughly with the appropriate concentrations of the nematicides used prior to sowing. Twenty pots were used for each treatment, each containing 5 seeds. Tap water was used for irrigation. For control, 20 pots were used in which tap water was used instead of the experimental solutions.

The study comprises measurment of the root length after 4, 6, 8, 10, 12 and 14 days after sowing taking the cotyledons as a starting point.

For mitotic study, the secondary roots of treated plants 12 days old were used. The roots were cut, fixed in Carnoy's fixative (1-3 acetic alcohol) for 24 hours then stored under refrigeration in 70% alcohol.

II. Laboratory experiments:

Vicia faba roots 4-days old (1.5 cm. in length) were dipped in varying concentrations of nemagon and nemacur (1, 10, 100 and 500 ppm) for 3, 6, 12 and 24 hours. After the fixed time, the roots were cut, fixed and stored as previously mentioned.

For recovery tests, another group of seedlings were taken out of the solutions after every time of treatment (3, 6, 12 and 24 hours), washed with running tap water then dipped in tap water for 24 hours. The recovered roots were cut, fixed as previously mentioned.

Observations were made from permanent Feulgen stained root tip squash preparations.

Mitotic index was calculated as the mean of dividing cells from 10 different root tips, for every treatment 10000 cell were counted.

Nuclear volume was estimated from a random sample of nuclei for each treatment and was calculated as

$$\frac{4}{3}\pi \left(\frac{\text{Length} + \text{Breadth}}{4}\right)^3 \quad \text{Bennet 1970}$$

Results

Root growth

Statistical analysis showed that the stimulatory effect of the two nematicides was not significant except for the last reading of the median nemacur dose, Figs. (1 and 2).

Cytological studies

Tables 1 and 2 show that the two nematicides affected the percentage of mitotic phases. Accumulation of prophases was obvious after treatment with field doses of the two nematicides. In the direct treatment, accumulation of prophases was observed after 6 hours treatment with 1 ppm nemagon.

Recovery experiments of nemagon 1 and 10 ppm tends to regain the normal percentage of the mitotic phases as the control, while that of nemacur deviates the percentage of phases away from their normal ones, Table (2).

Toxicity appeared after recovery in nearly all time intervals of 100 and 500 ppm nemagon and nemacur. Nemacur was more toxic than nemagon, toxicity starting earlier (after 6 hours 100 ppm).

Inhibition of mitotic index was observed after treatment with both field and laboratory doses of the two nematicides. After field doses, inhibition of mitotic index was more obvious after nemagon treatments, while after direct laboratory treatments nemacur affected the mitotic index severely.

After recovery, the mito-depressive effect of nemacur decreased except after the longer periods of treatment.

Table 3 shows that maximum field doses of nemagon and nemacur decreased the average volume of the nucleus.

An obvious decrease in nuclear volume was observed after 24 hours treatment with 1 and 10 ppm nemagon and nemacur direct and recovery treatments. A marked increase in nuclear volume was observed after nemacur treatment for 3 hours with 1 ppm direct and after 6 hours with 1 ppm and 3 hours with 10 ppm recovery.

After field doses, the total percentage of abnormalities increased with increase of concentration after nemagon treatment. On the other hand, it was not correlated with concentration after nemacur treatments.

After direct treatment with the two nematicides, the total percentage of abnormalities was not correlated with concentration. Table (5) shows that a maximum percentage of abnormal cells (57.1) was observed after recovery of 10 ppm 24 hours treatment.

Most of the abnormalities were restricted to metaphases except with minimum nemacur doses. Prophase has the least percentage of abnormalities with the two nematicides.

Abnormal prophases, Fig. (3) were observed in Vicia roots after treatment with nemagon field doses, Table (6) and were more pronounced after laboratory treatment reaching 42.2% after 6 hours with 1 ppm.

Stickiness was a prominent abnormality after treatment with the two nematicides with both field and laboratory doses. After field doses the percentage of stickiness was inversely proportional to the concentration.

Stickiness was more obvious after direct treatment with nemagon than nemacur. Fig. (4) shows sticky metaphase chromosomes.

The percentage of bridges was higher in nemacur than nemagon after treatment with field doses. On the other hand, nemagon brought about a higher percentage of bridges than nemacur after laboratory treatment, Tables(6 and 7), Figs.(5 and 6).

Laggards were a less dominent abnormality after both field and laboratory doses of the two nematicides, Tables (6 and 7), Fig. (7).

The disturbed type was the other dominent abnormality observed after treatment with the two nematicides. After nemagon field doses the percentage of types of spindle disturbances increased with increase of doses. Yet with nemacur maximum percentage of spindle disturbance was reached after the median dose. With laboratory treatment, nemacur induced a high percentage of disrupted spindles compared with nemagon, Tables (6 and 7), Fig. (8).

Fragmentation was a less common abnormality after both field and laboratory doses of the two nematicides.

Recovery treatment with 3h 1ppm nemacur showed that fragmentation was the only abnormality scored (100%), Table(7), Fig. (9) .

Micro and multinucleate cells, Figs.(10 and 11) were not a common abnormality in this investigation.

Discussion

The present results indicate that the two nematicides had no significant effect on the root growth of Vicia faba i.e. Vicia roots tolerate the effect of the nematicides

Mitotic observations proved that nemacur was more toxic than nemagon. Prophase accumulation which was more pronounced after nemagon field and laboratory doses than nemacur indicates delay in the spindle formation. However, recovery after nemagon treatment changed those percentages to the normal level.

Applying field doses of nemagon proved to be a mitotic poison decreasing the mitotic index more than nemacur, while after nemacur laboratory doses mitotic poisoning starts earlier and more severly. However, this mitodepressive effect of nemagon increased after recovery experiments while it decreased in case of nemacur recovery experiments.

Maximum nuclear volume reduction being induced 24 hours after treatment with both nematicides indicated that they might play a role in DNA synthesis (Kihlman 1971).

Most of the abnormalities scored after nematicidal treatment were at metaphase stage, ensuring that the two nematicides showed a stathmokinetic tendencies, yet they did not affect an increase in the mitotic index due to accumulation of cells at prophase and metaphase.

Prophase poisoning might be due to different changes in the readily dividing cells and this leads to a critical retardation of the course of mitosis. This abnormality was similar to that reported by Levan and Tjio (1948) after treatment with phenols, D'Amato (1950) using natural compounds, and Barthelmess and El-Kabarity (1966) using acetanilide.

Nemacour induced higher percentage of stickiness than nemagon with field doses, while after laboratory direct and recovery experiments nemagon brought about a higher percentage of stickiness.

After treatment with the higher concentrations of the two nematicides sticky bridges were observed. While after treatment with the lower concentrations the bridges formed might be due to either adhesion of chromosomes or exchanges and reunion of chromatid or subchromatid. Bose and Bose (1970) found bridges with or without fragments treating Tomato with colchicine, diethyl sulphate and triethylamine.

Lagging chromosomes were more common after nemagon treatment than nemacur field and laboratory doses. Laggards and fragments might give rise to micronuclei.

After short periods treatment, 24 hours recovery was sufficient for the total percentage of abnormalities and mitotic phases to regain their normal behaviour in both nematicides.

Nemacur harmful effects were repaired by recovery treatments than nemacur.

Summary

Vicia faba has been subjected to two types of treatments using different doses of nemagon and nemacur. Field

doses used in bean fields were tested and laboratory doses where the concentrations used were 1, 10, 100 and 500 ppm for 3, 6, 12 and 24 hours. For both nematicides recovery tests for 24 hours in tap water were carried out.

The two nematicides showed no significant effect on root growth of Vicia except for the median nemacur dose, where stimulation in root length took place.

Under field conditions, nemagon proved to be a mitotic poison more than nemacur. While under laboratory conditions the mito-depressive effect as well as induction of spindle disruption was more pronounced in nemacur.

A reduction of nuclear volume was recorded after longer hours of treatment of nearly all laboratory doses of both nematicides.

The most dominating abnormalities were :- stickiness, bridges, laggards, spindle disturbance, fragments and micronuclei.

The two nematicides used proved to be stathmokinetic agents.

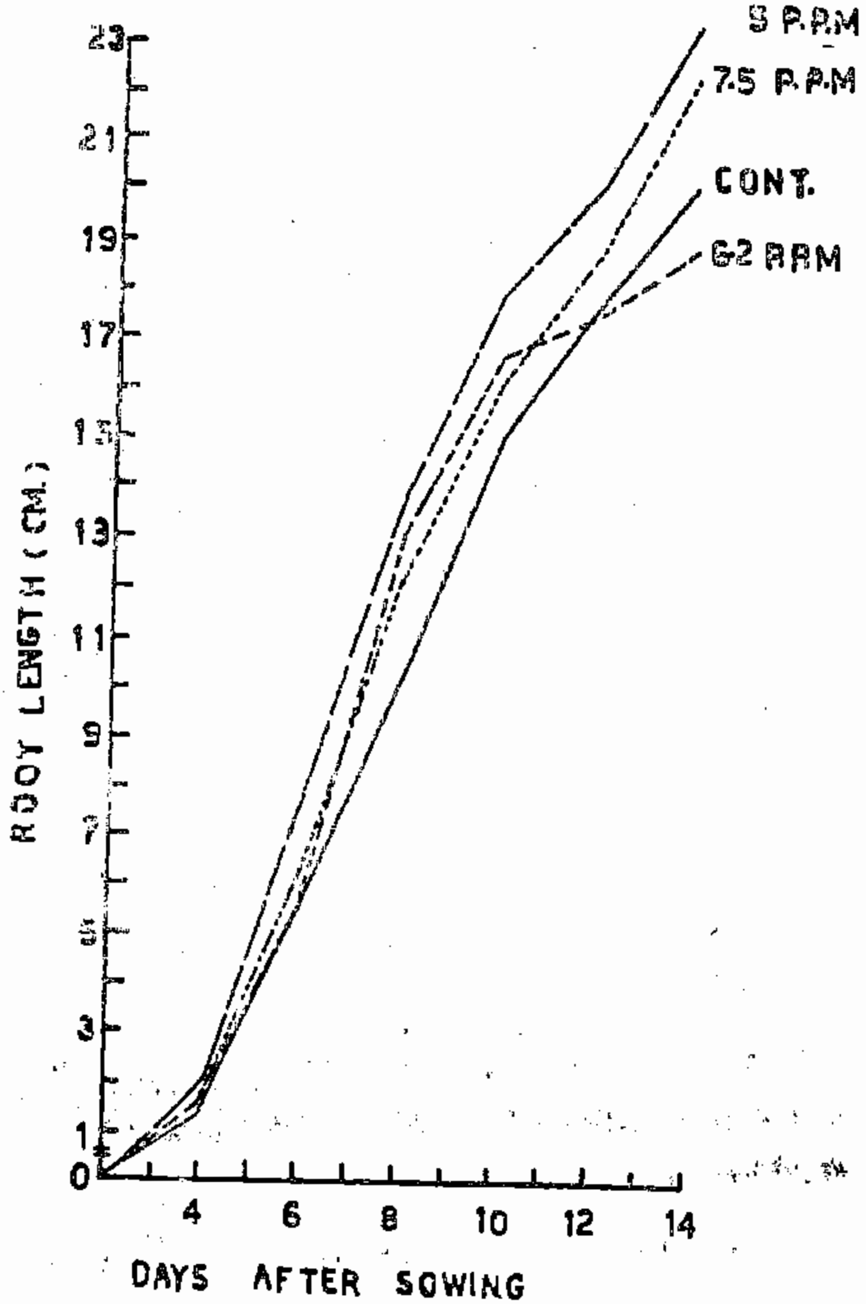

FIG. 1: ROOT LENGTH AS AFFECTED BY NEMAGON

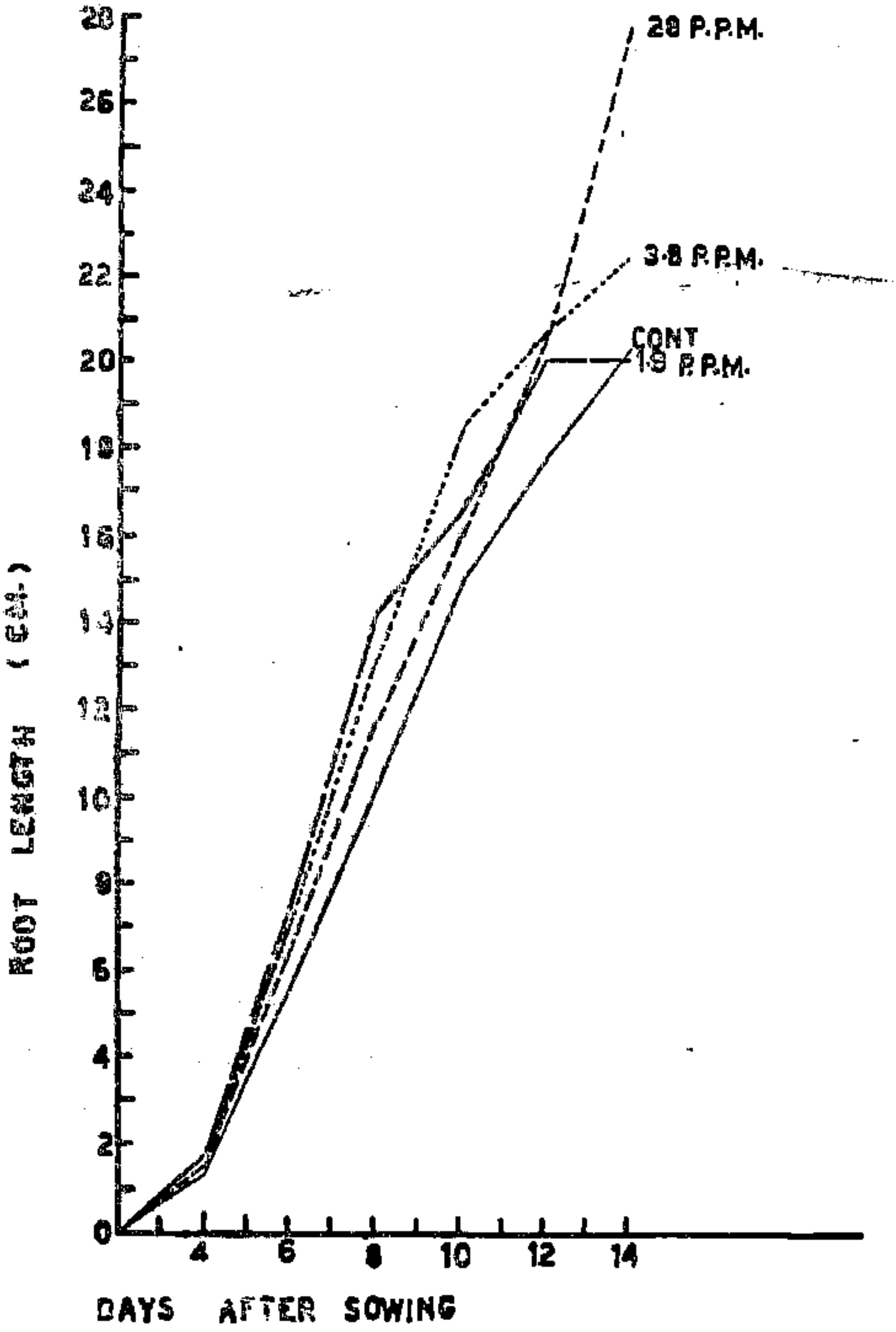

FIG: 2. ROOT LENGTH AS AFFECTED BY NEMACUR.

Table (1)

Percentage of Phases and mitotic index as effected by nemagon and Nemacur field doses

Type of treatment		Prophase	Metaphase	Ana-telophase	M
Control		40.8	16.50	4[illegible]	[illegible]
Nemagon	5.0	64.5	6.45	29.[illegible]	[illegible]
	6.2	66.6	10.40	22.9	[illegible]
	7.5	70.4	13.60	15.9	[illegible]
Nemacur	1.9	52.9	19.60	27.4	[illegible]
	2.8	47.2	15.20	37.5	[illegible]
	3.8	55.8	14.70	29.4	[illegible]

Table (2)

Percentages of phases and mitotic index after direct and recovery laboratory Nemagon and Nemacur treatments .

Type of	Prophase				Metaphase				Ana - telophase				Mitotic index			
	Nemagon		Nemacur		Nemagon		Nemacur		Nemagon		Nemacur		Nemagon		Nemacur	
	Direct	Recovery	D	R	D	R	D	R	D	R	D	R	D	R	D	R
Control	44.9	31.9	44.9	31.9	21.0	26.3	21.0	26.3	34.2	41.6	34.2	41.6	81	72	81	72
1 ppm 3h	41.9	26.3	43.0	45.2	20.4	28	17.3	10.0	37.7	45.6	40.8	35.8	64	57	46	53
6h	70	32.5	44.9	18.5	13.9	27.9	24.3	20.3	15.5	39.5	29.6	60.9	61	43	30	54
12h	32.9	27.7	25.6	12.8	23.4	22.2	35.5	25.6	43.9	50	30.7	61.5	66	94	44	39
24h	17.6	27.4	35.9	36.7	27.4	39.2	31.6	36.7	54.8	33.3	41.8	26.3	63.7	51	35	19
10 ppm 3h	50.3	22.0	47.5	41.8	14.1	30.5	14.4	28.1	20.8	47.4	37.9	40.0	54	59	42	59
6h	51.1	34.1	41.3	24.5	21.8	19.5	20.4	29.2	25.5	64.3	35.2	46.7	36	41	38	62
12h	30.4	34.7	24.2	28.8	26.6	28.6	32.1	26.9	45.6	39.1	43.6	44.8	58	69	38	52
24h	18.4	32.7	24.1	30.0	27.2	50.8	30.1	45.0	84.4	16.3	38.1	25.0	50	61	38	20
100 ppm 3h	39.0	18.1	57.8	62.7	32.7	30.1	19.6	9.8	24.8	39.6	24.7	27.4	64	53	51	51
6h	56.3	Toxic	Toxic		16.4	Toxic	Toxic		27.2	Toxic	Toxic		54	Toxic	Toxic	
12h	60.5	25.3	Toxic		18.2	34.9	Toxic		19.2	38.0	Toxic		26	63	Toxic	
24h	Toxic	Toxic	Toxic		Toxic	Toxic	Toxic		toxic	toxic	Toxic		toxic	toxic	Toxic	
500 ppm 3h	57.6	Toxic	Toxic		24.7	Toxic	Toxic		Toxic	17.5	Toxic		Toxic	36	Toxic	
6h	Toxic	Toxic	Toxic		Toxic	Toxic	Toxic		Toxic	Toxic	Toxic		Toxic	Toxic	Toxic	
12h	Toxic	Toxic	Toxic		Toxic	Toxic	Toxic		Toxic	Toxic	Toxic		Toxic	Toxic	Toxic	
24h	Toxic	Toxic	Toxic		Toxic	Toxic	Toxic		Toxic	Toxic	Toxic		Toxic	Toxic	Toxic	

Table (3)

Nuclear volume (u^3) as affected by Nemagon and Nemacur

Field		Laboratory					
				Nemagon		Nemacur	
				Direct	Recovery	Direct	Recovery
Control	2178.2	Control		3580.08	3580.08	3580.08	3580.08
Nemagon P.P.m		1P/P.m	3h	3580.08	3930.0	6061.1	4431.5
5	1981.5		6h	4697.5	4055.9	2655.9	5260.5
6.2	2475.1		12h	4973.6	3694.5	3580.0	3356.7
7.5	1371.8		24h	1494.5	1146.7	1761.4	1692.1
Nemacur P.P.m		10 ppm	3h	3580.00	4834.1	1761.4	5711.5
1.9	2219.1		6h	4431.5	4302.5	3811.0	3358.7
2.8	1981.5		12h	4176.0	3246.5	2845.5	2943.6
3.8	1632.1		24h	1624.4	2943.6	1200.6	814.7
		100 P.P.m.	3h	3811.01	[illegible]	3930.0	4697.5
			6h	3811.03	2655.9	2268.8	Toxic
			12h	4302.5	3146.6	1905.3	Toxic
			24h	Toxic	Toxic	Toxic	Toxic

Table (14)

Total percentage of abnormalities and distribution of percentages of abnormalities in the different mitotic phases after treatment with field doses of Nemagon and Nemacur.

Type of treatment	Total percentage of abnormalities.	Prophase	Metaphase	Ana-telophase
Control	2.2	-	-	-
Nemagon ppm.5	7.6	0	57.0	43.0
6.2	6.7	9.5	52.3	38.0
7.5	11.9	11.5	69.2	19.2
Nemacur ppm.1.9	20.3	0	66.6	33.9
2.8	14.8	0	70.4	29.6
3.8	16.5	0	42.8	57.1

Table (5)

Total percentage of abnormalities and distribution of percentages of abnormalities in the different mitotic phases after treatment with lab doses of Nemagon and Nemacur

Type of treatment	Nemagon								Nemacur							
	Direct			Recovery			Total % of ab.		Direct			Recovery			Total % of ab.	
	Proph	Meta	ana-t-	proph.	Meta	ana-telo	D	R	proph.	meta	Ana-telo.	proph.	meta	ana-telo	D	R
Control							2.9	1.4							[illegible]	14
1 ppm 3h	6.5	60.8	32.6	19.7	48.4	31.8	14.4	2.8	[illegible]	25.5	65.7	0	0	100	[illegible]	3
6h	55.5	33.3	11.1	20.0	0	60.0	18.4	4.7	5	65.	30	10.5	52.6	36.8	10.5	7
12h	6.0	54.5	39.4	9.0	42.4	48.4	12.5	12.5	0	72.2	[illegible]1.7	11.1	38.8	50	[illegible]	11.8
24h	0	46.4	33.5	0	34.0	15.9	10.9	17.7	7.1	50	42.6	6.2	50.0	43.7	[illegible]	27.5
10 ppm 3h	0	55.5	14.4	33.3	5.6	72.2	[illegible].6	[illegible]	[illegible]3	[illegible]	[illegible]6.6	18.1	[illegible].2	54.5	[illegible]0.1	4.5
6h	18.4	55.2	26.3	17.[illegible]	42.8	39.2	[illegible]2.0	16.6	0	[illegible]	40.0	[illegible].1	90.6	6.2	5	13
12h	4.6	53.4	41.8	13.5	67.6	18.9	14.5	[illegible]	0	67.2	[illegible].6	9.5	76.2	14.3	[illegible]	10.5
24h	4.7	23.8	71.4	1.4	77.9	20.5	15.0	57.1	[illegible]	38.[illegible]	[illegible]0	9	77.3	13.6	9	26.3
100 ppm 3h	0	82.5	17.4	24.0	46.0	26.0	[illegible].4	[illegible]	23.0	[illegible].1	30.7	4.7	90.4	4.7	[illegible]0.5	6.2
6h	35.0	37.5	32.5	Toxic	Toxic	Toxic	17.3	Toxic	Toxic	Toxic	Toxic	Toxic	Toxic	Toxic	Toxic	Toxic
12h	4.5	45.0	50.0	32.5	56.9	37.5	21.2	Toxic	Toxic	"	"	"	"	"	"	"
24h	Toxic	Toxic		Toxic		Toxic	Toxic	Toxic	Toxic	"	"	"	"	"	"	"

Table (6)

Percentages of different types of abnormalities after treatment with field doses of Nemagon and Nemacur

Type of treatment	Types of abnormalities					
	Abn.proph.	Stick.	Bridges	Lagging	Sp. dist.	Frag.
Nemagon ppm 5.0	0.0	17.3	34.7	0	47.5	0.0
6.2	9.5	4.7	9.5	19.0	57.0	0.0
7.5	11.5	0.0	11.5	3.8	72.9	0.0
Nemacur ppm 1.9	0.0	42.5	33.3	0.0	24.0	0.0
2.8	0.0	16.6	18.5	11.1	49.9	3.7
3.8	0.0	5.3	50.0	3.5	37.4	3.5

Table (7)

Percentages of different types of abnormalities after laboratory Direct and Recovery treatment with nemagon and nemacur.

Type of treatment	Types of abnormalities																							
	Abn. proph.				Stickiness				Bridges				Lagging				Sp. dist				Frag.			
	Nemagon		Nemacur		Nemagon		Nemacur		Nemagon		Nemacur		Nemagon		Nemacur		Nemagon		Nemacur		Nemagon		Nemacur	
	D	R	D	R	D	R	D	R	D	R	D	R	D	R	D	R	D	R	D	R	D	R	D	R
1 p.p.m 3h	6.5	9.7	5.0	—	39.1	58.5	5	—	19.5	12.1	—	—	10.7	4.8	15	—	21.9	7.3	700	—	2.1	12.1	5	100
6h	42.2	—	5.7	—	33.3	20.0	—	15.7	4.4	—	25.7	—	4.4	80.0		10.5	11.0	—	31.3	57.8	4.0	—	17.1	15.7
12h	3.0	3.0		11.1	21.2	15.1	45.6	27.7	18.1	3	6.5	22.2	21.1	24.1	2.1	—	36.3	51.4	36.3	27.7	—	3.0	8.6	11.1
24h	—	—	7.1	6.2	7.1	56.8	21.4		32.1	2.2	21.4	—	3.5	4.5	7.1	—	50.0	36.2	28.5	75.0	7.1	—	14.2	18.7
10 p.p.m 3h	—	27.7	2.3	—	44.4	22.2	23.0	9.0	11.1	11.0	21.4	—	11.1	5.5	7.1	18.1	22.2	27.7	30.8	18.0	11.1	5.5	35.6	[illegible]
6h	5.2	7.1	—	—	52.6	39.2	—	15.6	15.7	—	—	—	7.8	31.5	10.0	—	18.3	23.1	50.0	34.2	—	—	—	—
12 h	2.3	8.1	—	—	30.2	24.3	29.5	14.6	—	8.1	16.3	9.5	13.6	8.1	1.6	4.7	44.1	38.6	50.8	71.4	4.6	2.7	1.6	—
24 h	—	1.4	—	—	4.7	29.4	5.6	86.3	40.4	4.4	16.6	9.0	21.3	5.8	—	—	26.1	52.9	38.8	4.5	7.1	5.8	38.8	—
100 p.p.m 3h	—	—	23.7	4.7	2.3	56.0	25.0	9.5	4.6	12.0	—	—	6.8	4.0	—	—	84.8	24.0	44.1	35.0	1.1	4.0	7.6	—
6h	21.4	Toxic			23.8	Toxic	Toxic		9.5	Toxic			1.8		Toxic		23.4	—	—	—	4.7	—	—	—
12h	—	1.7	Toxic		45.4	64.2	Toxic		40.9	5.3	Toxic		45	7.1	Toxic		9.0	19.6	Toxic		—	1.7	Toxic	
24h	Toxic		Toxic		Toxic		Toxic		Toxic		Toxic		Toxic		Toxic		Toxic		Toxic		Toxic		Toxic	

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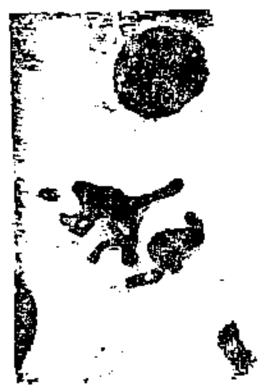

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Plate I

Fig. 3 : Irregular prophase after treatment with nemagon 10 ppm 24 hours and recovery.

Fig. 4 : Sticky metaphase after treatment with 100 ppm 6 hours nemagon.

Fig. 5 : Sticky bridge at anaphase after treatment with maximum nemacur field dose 3.8 ppm.

Fig. 6 : Chromatid bridge after treatment with maximum nemacur dose 3.8 ppm.

Fig. 7 : Lagging chromosome (two chromatids) after treatment with nemagon 10 ppm for 24 hours and recovery.

Fig. 8 : Disturbed metaphase after treatment with maximum nemagon.

Fig. 9 : Metaphase breakage after treatment with nemagon 10 ppm 24 hours and recovery.

Fig.10 : Multinucleate after treatment with nemagon 10 ppm 24hours and recovery.

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