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FINAL REPORT
ON
EVALUATION OF JUVENILE HORMONE NKI-35120 IN PAKISTAN

INTRODUCTION:

In view of increasing hazards of pesticide use to human being, wild life, natural enemies of insects and other living organisms and their environment the United Nations Industrial Development Organization (UNIDO), Vienna, Austria provided technical assistance to Plant Protection Institute of the Hungarian Academy of Sciences, Budapest, Hungary for development of non-toxic anti-insect agents under the Project No. DP/HUN/86/006. The project was to develop juvenile and anti-juvenile hormone compounds which are known for their property of bio-chemical and behavioral changes in the body of insects; and supposed to be environmentally safer compared to the conventional pesticides. These compounds are categorized on the basis of their mode of action as follows:

1. Compounds affecting biosynthesis or function of hormones regulating metamorphosis (juvenile hormone mimics, anti-juvenile hormones, neurohormones, ecdysone agonists).
2. Compounds inhibiting formation of insect cuticle.
3. Compounds interfering with insect behavioural patterns (pheromones, antifeedants, oviposition deterrents etc.).

Although these chemicals are highly selective to insects, and their practical application created problems limiting their use for specific fields and necessitating further research. The project was extended to second phase in view of identification of two potential lead compounds which had juvenile hormone activities inhibiting spiracle and crochet formation. This warranted further field trials on cotton in the developing countries.

The Directorate of Research (Crop Protection), Pakistan Agricultural Research Council (PARC), Islamabad, Pakistan was selected to undertake the trials. Mr. Paul Scheltes of Duphar B.V. Netherlands visited PARC on Monday the 21 May, 1990 to follow-up the agreed testing of the above compounds. Detailed discussions with regard to conduct of trial locations, lay-out plan, spray interval and efficacy evaluation methods alongwith frequency of the evaluation were held. M/s Duphar Limited representative promised to send the samples urgently to start trials during fist fortnight of June because white fly

infestation had already started. The detailed agreed protocol for testing the subject compound in Pakistan during 1990 were received in Pakistan on 21 June, 1990 through a telefax message (Annex-I). However the despatch of test compound samples from Netherlands was delayed until 04 August, 1990 (Annex-II); and they were received in Pakistan on 15 August 1990. Director of Research (Crop Protection) PARC, Islamabad immediately travelled to both the test locations at Faisalabad (Punjab) and at D.I. Khan (NWFP) on 20-22 August 1990.

Since the despatch of sample from Netherlands was delayed therefore the testing of samples against different white fly stages alone was not possible because the bollworms infestation had already set in. Detailed discussions were held on protocol for testing the juvenile hormone experimental compound (NKI-35120) alongwith Fenoxycarb WP-25 and a local check polytrin-C (a combination of profenofos 400 gram + cypermethrin 40 gram/liter) with the scientists at both the locations. Fenoxycarb was inserted as direct standard for NKI-35120, because both are juvenoids. Fenoxycarb is already commercially available on the European market, trade-name: insegar. Polytrinc-C was used as local standard because it is widely used pesticide against sucking insects and bollworm species on cotton in Pakistan.

MATERIAL & METHODS:

Crop:	Cotton variety NIAB-85
Location:	Two (Faisalabad and D.I. Khan)
Target insects:	Tobacco white fly (Bemisia tabaci)
	Bollworms (Earias spp.; Heliothis armigera; Pectinophora gossypiella)
Plot size:	81 meter square
Replications:	Four
No. of sprays:	One (originally 3 sprays were scheduled but due to delay in receipt of samples only one spray was possible/conducted)
Spray interval:	Since only one spray was done, the originally scheduled intervals could not be followed.
Spray Liquid:	750 l/hectare

	Treatment	Test dosage	
		g a.i./acre	ml (g) product/acre
1.	NKI-35120 EC-25	20	80 ml
2.	NKI-35120 EC-25	40	160 ml
3.	NKI-35120 EC-25	80	320 ml
4.	Fenoxycarb WP-25	40	160 g
5.	Fenoxycarb WP-25	80	320 g
6.	Fenoxycarb WP-25	160	640 g
7.	Polytrin-C EC-400	200 Profenofos + 20 Cypermethrin	500 ml
8.	Untreated check	-	-

Time of spray: Only one spray could be carried on 27th of August 1990 at both the locations which was mainly directed against bollworms, because whit fly population was on decline.

Method of spray: Spray was carried out in the morning hours with a knapsack sprayer which is manually operated. A good plant coverage was given.

Method of evaluation: White fly: For evaluation of white fly population 10 plants randomly distributed in every plot were selected for each assessment. Separate counts of the number of nymphs and adults on 5 but at least one previously marked leaf/plant were taken in the early morning when adults were less active. The percentage of white fly normal nymphs and adults in the field on twelveth day was recorded. Observations for phytotoxicity were also taken.

Bollworm: Two hundred squares/ bolls were randomly taken from each plot; and number of live and dead larvae was counted. The percentage of normal adults emerging from pupae collected from the field was determined in the lab. Observations for phytotoxicity were also taken.

Time and frequency of evaluation:

Assessment	Insect	Time
1	White fly	Immediately before spray
2	White fly	Three days after spray
3	White fly	Seven days after spray
4.	Bollworms	Immediately before spray
5.	Bollworms	Three days after spray
6	Bollworms	Seven days after spray
7.	Bollworms	Fourteen days after spray
8.	Bollworms	Twenty one days after spray

RESULTS:

The data collected was converted to percent mortality/abnormality which is presented in Table-I and II. The rate of natural mortality (%) for white fly nymphs ranged between 2-5% at Faisalabad while it was higher (4-13%) at D.I. Khan. Similarly the rate of natural mortality (%) of adults of white fly was between 5-9% at Faisalabad and 4-13% at D.I. Khan. The range of mortality (%) of both nymphs and adults at D.I. Khan was also higher in the untreated check as compared to Faisalabad. This shows that some biotic or abiotic factors were suppressing the population build-up of whit fly at D.I Khan under natural conditions.

The rate of percent mortality both for white fly nymphs and adults at both the locations (Faisalabad and D.I. Khan) was much below that by commonly used pesticide (Polytrin-C) against these insects which was up to 100%. However, it is evident from the data that NKI-35120 EC-25 was more effective in killing both the nymphs and adults of white fly than Fenoxycarb at both the locations. The data also shows that the test dosage of 80 g a.i. of NKI-35120 EC-25 was more effective compared to the other two dosages of 20 g a.i./acre and 40 g a.i./acre.

This suggest that we may repeat the trials for determination of effective and economical dosage of the test compound. The suggested dosage is 50 g a.i./acre and 100 g a.i./acre as against 100 g a.i. and 180 g a.i. of Fenoxycarb WP-25.

The rate of normal white fly emergences (%) was very low (16-19% for nymphs and adults respectively at Faisalabad and 20-24% for nymphs and adults respectively at D.I. Khan) for NKI-35120 EC-25 applied @ 80 g a.i./acre as compared to other dosages of test compound as well as Fenoxycarb WP-25.

The results presented in Table-II indicate that the cotton bollworm larval mortality (%) after 21 days was 67% and 65% at Faisalabad and D.I. Khan, respectively which is much lower compared to Polytrin-C (Faisalabad 85% and D.I. Khan 81%). However, normal adult emergence percentage was also very low (23%) in the NKI-35120 EC-25 applied @ 80 g a.i./acre compared to Polytrin-C (95%) and untreated check 93%. This shows that initial kill by both the juvenile hormones was low compared to Polytrin-C (local check) but the normal adult emergence is also lower in the former compounds compared to the later. This helps in checking the population build-up of the insect, if juvenile hormone are used.

CONCLUSION:

We can concluded that the rate of bollworms and white fly mortality (%) although is low at both the locations compared to Polytrin-C but normal adult emergence rate is also very low. This indicates that test compund is quite effective against both the white fly as well as cotton bollworms in checking their population and will be more economical in the long run.

Since the despatch of sample was extremely delayed when the attack of bollworms had setin and population of white fly was on decline, therefore, we can not draw meaningful conclusions. The protocol needs to be repeated.

Table-1: White fly mortality (%) and normal adult emergence (%)

Location	Treatment	Test dose g.a.i./acre	Before spray		After spray				Normal Adult Emergence (%)	
					3 days		7 days			
			N*	A**	N	A	N	A	N	A
Faisalabad	1. NKI-35120 EC-25	20	3	7	15	23	19	27	47	36
	2. NKI-35120 EC-25	40	4	5	23	27	37	49	33	31
	3. NKI-35120 EC-25	80	2	8	41	52	58	67	16	19
	4. Fenoxycarb WP-25	40	3	5	17	22	18	24	41	43
	5. Fenoxycarb WP-25	80	5	7	27	35	33	42	34	29
	6. Fenoxycarb WP-25	160	2	6	31	42	45	53	21	20
	7. Polytrin-C EC-400	200	4	9	93	100	97	100	78	89
	8. Untreated check	-	3	8	9	4	7	5	92	89
D.I. Khan	1. NKI-35120 EC-25	20	7	12	22	29	31	30	43	38
	2. NKI-35120 EC-25	40	9	14	24	33	27	39	30	33
	3. NKI-35120 EC-25	80	4	10	43	53	54	67	24	20
	4. Fenoxycarb WP-25	40	8	12	21	23	22	27	31	30
	5. Fenoxycarb WP-25	80	7	11	23	31	37	41	23	27
	6. Fenoxycarb WP-25	160	13	17	35	51	45	59	18	23
	7. Polytrin-C EC-400	200	8	14	100	100	100	100	91	93
	8. Untreated check	-	6	12	10	10	11	15	94	95

*N= Nymph **A= Adult

Table-II: Cotton bollworms larval mortality and normal adult emergence

Location	Treatment	Test dose g a.i./acre	Larval mortality (%) Before Spray	After spray (days) 3	7	14	21	Normal adult emergence (%)
Faisalabad	1.NKI-35120 EC-25	20	4	10	19	27	36	49
	2.NKI-35120 EC-25	40	2	20	31	45	51	37
	3.NKI-35120 EC-25	80	3	29	51	59	67	23
	4.Fenoxycarb WP-25	40	5	9	17	20	26	59
	5.Fenoxycarb WP-25	80	3	14	27	29	34	43
	6.Fenoxycarb WP-25	160	2	19	26	41	50	32
	7.Polytrin-C EC-400	200	4	71	77	82	85	87
	8.Untreated check	-	5	3	4	3	6	89
D.I. Khan	1.NKI-35120 EC-25	20	3	8	13	19	29	46
	2.NKI-35120 EC-25	40	5	13	27	29	47	35
	3.NKI-35120 EC-25	80	10	21	42	53	65	21
	4.Fenoxycarb WP-25	40	7	8	13	19	26	53
	5.Fenoxycarb WP-25	80	5	11	25	32	48	37
	6.Fenoxycarb WP-25	160	7	21	39	46	55	21
	7.Polytrin-C EC-400	200	3	67	69	73	81	95
	8.Untreated check	-	5	6	4	8	7	92

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Dear Mr. Baloch,

It was quite a pleasure to have been able to personally meet you in Islamabad on 21st May.

Based on our discussion on the lay-out of the experiments, I have now made a protocol of which a copy is enclosed. I like to specifically mention a few items:

1. due to a limited sample size I have reduced the number of locations to two only.
2. I have inserted fenoxycarb as a direct standard for NKI-35120, because both products are juvenoids. Fenoxycarb is already commercially available on the European market, tradename: Insegar. Next to this standard, I would like you to compare NKI-35120 with one local standard product against whitefly and one against bollworms.
3. 3 sprays will be applied to cover both whiteflies (initially) and bollworms (later during season).
4. the spray interval between 2nd and 3rd spray is given (2 weeks). However, I left the spray interval between 1st and 2nd spray open, because of its dependence on the nymphal developmental time: 2nd spray should preferably be directed against the last nymphal stage, which appearance is dependant on the local climatic conditions.
5. although I have asked to record nymphal abnormalities for whiteflies, this may be [illegible] possible. See what can be achieved. Abnormalities in bollworm larvae/pupae will [illegible]

In general, I would like to stress that this protocol should be considered as a guideline. Please be practical in its implementation. Any suggestions for changes are welcomed. If further contact on the protocol is required, I would like to inform you that I will have my holidays from [illegible] July.

Meanwhile I have asked our forwarding dept. to send you the following samples:
2 bottles of 150 ml NKI 35120 EC-25
2 containers of 300 g fenoxycarb WP-25
We expect these samples will reach you (by airfreight) end June/early July.

Hoping to hear your comments soon, I remain.

Yours sincerely,

PROTOCOL FOR TESTING NKI-35120 IN PAKISTAN 1990

Crop: cotton
Locations: 2
Target insects: *Bemisia tabaci* - tobacco whitefly
Earias spp. - spotted bollworm
Heliothis armigera - American bollworm
Pectinophora gossypiella - pink bollworm
Plot size: 1/50 A (81 m²)
Replicates: 4
No. of sprays: 3 (T_1, T_2 and T_3)
Spray interval: 2-3 weeks interval
Spray liquid: 250-300 l/A

Compounds	Test dosages	
	g a.i./A	ml (g) product/A
1. NKI-35120 EC-25	20	80 ml
2. NKI-35120 EC-25	40	[illegible]
3. NKI-35120 EC-25	80	[illegible]
4. fenoxycarb WP-25	40	[illegible]
5. fenoxycarb WP-25	80	[illegible]
6. fenoxycarb WP-25	160	[illegible]
7. Local standard product for whitefly		[illegible]
8. Local standard product for bollworm		[illegible]
9. Untreated control		

Mode of action of test compound

The test compound NKI-35120 is a non-neurotoxic insecticide with a strong juvenile hormone activity. Due to this quality metamorphosis to the adult stage is highly inhibited which results in morphogenetically deformed insects (larval-pupal and pupal-adult intermediates) and death of last larval and pupal stages.

The compound also induces ovicidal effects and shows interference with the moulting of early instar larvae in certain insect species. NKI-35120 acts by contact and ingestion.

Time of spraying

First spray should be directed against whiteflies before bollworms are [illegible] First time of spraying is at an early stage of whitefly development when the whitefly population consists of eggs and first instar nymphs.

Second spray should be applied when the majority of the whitefly population has reached the 2nd nymphal stage i.e. 2-3 weeks after the 1st spray. This spray is directed [illegible]

Third spray to be applied 2 weeks after 2nd spray. This spray is mainly directed [illegible]

Evaluation method

Whitefly:

- For evaluation select at least 10 plants/plot, randomly distributed in every plot.
  For each assessment separately count the number of eggs (is possible), nymphs and adults on five but at least one previously marked leaves (leaf) plant. If possible the under surface of the leaves should be carefully scrutinized without disturbing the insects. Best time for inspection is early morning, when adults are less active.
- Record nymphal abnormalities (if possible).
- Record phytotoxicity if any.

Bollworms:

- Count the number of live larvae on 200 squares or bolls taken randomly from each plot.
  Determine % infested squares or bolls per plot.
- Record larval/pupal abnormalities.
- Record phytotoxicity if any.

Time and frequency of evaluation

The effectiveness of the products are assessed 10 times according to the following time schedule:

Assessment no.	time
1	Immediately before T_1
2	T_1 + 3 days
3	T_1 + days *
4	immediately before T_2
5	T_2 + 3 days
6	T_2 + days *
7	immediately before T_3
8	T_3 + 3 days
9	T_3 + 12 days
10	T_3 + 21 days

* depending on rate of nymphal development.