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TRANSLOCATION STUDIES WITH MONOCROTOPHOS IN PUMPKIN PLANTS BY GRANULE IMPREGNATION TECHNIQUE

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Abstract. Studies were undertaken to investigate various aspects of translocation of Monocrotophos (Azodrin) residues in pumpkin plants following stem application (by granule impregnation). Larvae and adult beetles of *Epilachna dodecostigma* Muls. were used as test insects for bioassay. Results indicate that rapid translocation of insecticide occurs throughout the plant up to a distance of 24 ft. Granules of higher concentration were noted to translocate insecticide residues comparatively slowly compared with lower concentration granules. Exposure of insects to treated plants for 24 hr controlled the pests effectively only up to 5 days whereas 48–hr exposure provided good kill up to 11 days. It was also observed that younger plants were comparatively more efficient in translocation than older plants.

Various laboratory and field methods of systemic insecticides application are available but stem application by banding or injection into the plant system has, of recent, been demonstrated by various, workers to be much more efficient than soil or foliar application.[1,3–8] The present studies were undertaken to investigate an altogether new aspect of systemic insecticide application by impregnating granules into the plant stem through a small slit. For this work Monocrotophos (Azodrin) was selected and various aspects of translocation of its residues inside pumpkin plants were studied by using as test insects, adults and larvae of *Epilachna*, a serious pest of pumkin. Chemical tests of treated plant leaf extracts using thin layer chromatography were carried out to draw a rough correlation between the amounts of insecticide residues present and insect kill obtained.

The various aspects of translocation of Monocrotophos studied in these experiments were as follows: (1) direction of movement in the plant, (2) distance and rate of translocation, (3) residual toxicity (4) concentration effects, (5) effect of age of the plant on distribution of insecticide; and (6) chemical estimation of residues present in the leaves of treated plants.

Material and Methods

Rearing of Insect. The *Epilachna* beetles were reared on fresh leaves of pumpkin in the laboratory in 5-lb glass jars sealed with muslin cloth. Eggs were laid in batches of 20–40. Newly laid eggs are bright yellow becoming whitish near hatching. After 3–4 days incubation period small bright yellow hairy larvae less than 2 mm in length, hatched out. Two days later they moult to start the second larval stage. During the whole larval period (8–10 days) at 30–50°C four moultings occurred at 2–3 days intervals. The pupal stage lasts for 3–4 days whereafter a bright yellow beetle emerges. The second stage larvae, about 2–3 mm long, were used for this investigation.

Insect Cages. Small cages (Fig. A) were used to maintain insects on the plant. Each cage consisted of a glass tube (1.5×2 cm) attached at one end to a 3.5–cm square piece of sponge rubber (7 mm thick) with a hole in the centre. The other end of the tube was secured with a piece of muslin to allow ventilation. Another square piece of sponge rubber (3.5 cm square) was glued to the lower arm of a wire clip. Ten larvae were placed on a leaf and the sponge end of the glass tube was positioned over them. Subsequently, the sponge of the wire clip was positioned under the leaf corresponding to the sponge-end of the glass tube and the whole assembly was attached the leaf by securing both arms of the clip with a rubber band.

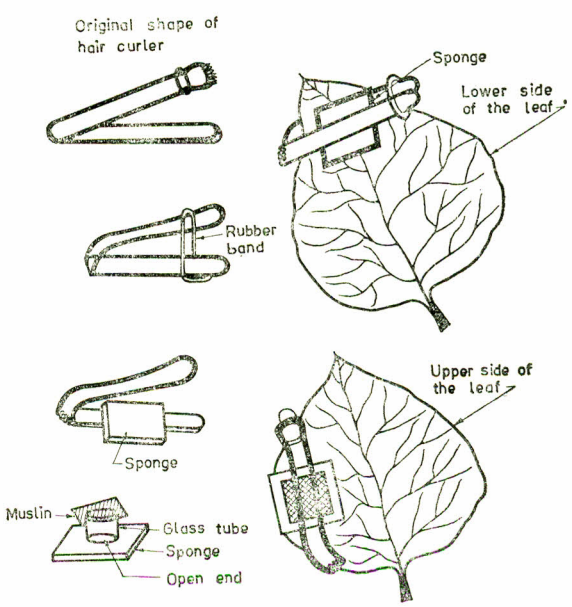

Fig. A. Insect cage used in the experiment.

Monocrotophos Granules. Granules of the insecticide (12, 24 and 48%) were prepared with Fuller's earth, an indigenous diluent found in abundance in Pakistan. A slurry of the insecticide and Fuller's earth was prepared with acetone and a quantity of water. The paste thus prepared was spread over a glass plate in a thin layer of about 2 mm depth. When the mixture had dried small pieces of about 3×2 mm were cut with the help of a sharp knife and stored in glass bottles.

Application of Granules. In order to apply the granules, a longitudinal slit of about 3 cm was made near the base of the stem of the pumpkin plant and 100 mg of the granules, irrespective of their concentration, were placed inside the slit by holding the cut portions apart with forceps. Three concentrations were used, but the quantity of granules applied to each plant was kept constant to study relative concentration effects.

Experimental

To investigate the aforementioned aspects of Monocrotophos translocation and persistance in pumpkin plants, each experiment was repeated 2–3 times using 2-day old larvae according to the method described earlier. The pumpkin plant which is basically a creeper, was especially selected for these experiments because of its long and hollow stem. The larvae were confined (in small cages) to the treated plant for four different periods, i.e. 1, 4, 24 and 48 hrs. Thereafter, they were transferred to small tubes (5.5×2.5 cm) and fed on fresh pumpkin leaves. After exposure, the larvae were kept under observation for a further period of 24 hrs in order to assess mortality. Adult beetles were placed in petri dishes of 9-cm dia and fed on leaves taken from the treated plants at similar intervals after treatment and the percentage mortality was recorded.

Experiment 1

Direction of Movement. Studies were made to determine the direction in which the insecticide moves inside pumpkin plant. For this purpose insecticide granules were placed in a slit made on the main stem near the middle of the plant and insect larvae were attached to leaves on either side of the slit. It was obsrved that no mortality occurred below the slit even after 24 hrs among insects attached to the plant at 2, 6, or 12 ft from the site of application. No downward movement of the insecticide was noted. However, effective concentrations were detected up to a distance of 24 ft ahead of the slit causing up to 90% mortality on the fourth day, on main stem as well as on the side branches of the pumpkin plant (Fig. 1).

Experiment 2

(a) *Distance and Rate of Translocation.* Through bioassay the presence or absence of effective insecticide concentrations were determined at various distances from the point of entry into the plant system. Simultaneously, tests were conducted to find out the time taken by the insecticide to translocate over various distances from the slit. For this purpose, insect larvae were kept attached to the treated plants on the main stem as well as on side branches for 1, 4, 24 and 48 hr continuously and mortality counts were taken thereafter. Adult beetles were fed on leaves taken from treated plants after similar periods. The results obtained indicated that distribution of the insecticide was uniform and that the insecticide sustains a lethal concentration throughout the length of the plant (Figs. 2 and 3).

(b) *Residual Toxicity.* Combined with the tests conducted in respect to distance and rate of translocation of Monocrotophos, experiments were arranged to assess the effect of length of insect exposure, both larvae and adutls, to treated plants at three distances from the point of insertion of insecticide

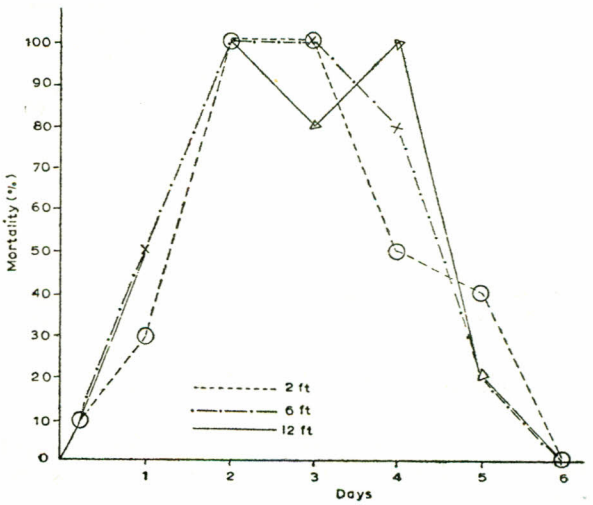

Fig. 1. Direction of movement in the plants.

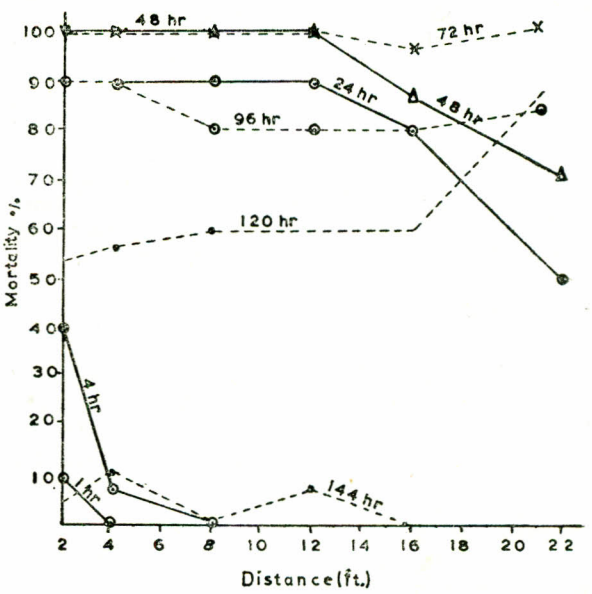

Fig. 2. Distance and rate of translocation.

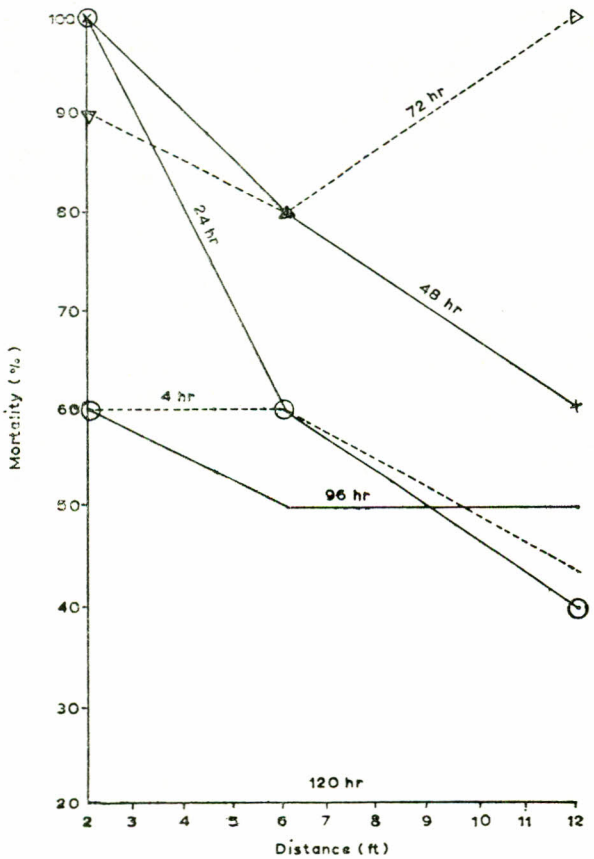

Fig. 3. Distance and rate of translocation.

granules. For this purpose larvae were kept attached to the treated leaves continuously or 24 and 48 hr and mortality counts were taken. Fresh batches of larvae were exposed to treated plant leaves every 24 and 48 hr. Observations were continued until mortality dropped to nil. The same procedure was adopted for adults insects but instead of confining them on treated plants they were fed on leaves plucked from tred teaplant and the mortality was recorded. It was noted that the insecticide controlled the insects up to 11 days when they were exposed to the treated plants for 48 hrs continuously (Figs. 4 and 5). In comparision, good kill could be obtained only up to 5 days when insects were kept in contact with the treated plants for only 24 hr (Figs. 4 and 5).

Experiments 3

Effect of Concentration of Granules on Insect Kill and Persistance. For these tests 12, 24 and 48% granules of Monocrotophos were used. Results indicated that the residual effect increases with the use of granules of higher concentration. Translocation of Monocrotophos from granules of higher concentration was shown to be slower than from granules of lower concentrations (Figs. 6 and 7). Possible explanation of this phenomenon may concern solubility coefficient limits of the insecticide in the plant.

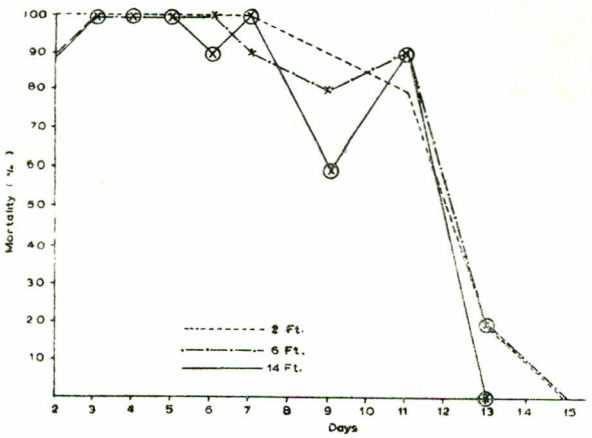

Fig. 4. Residual toxicity.

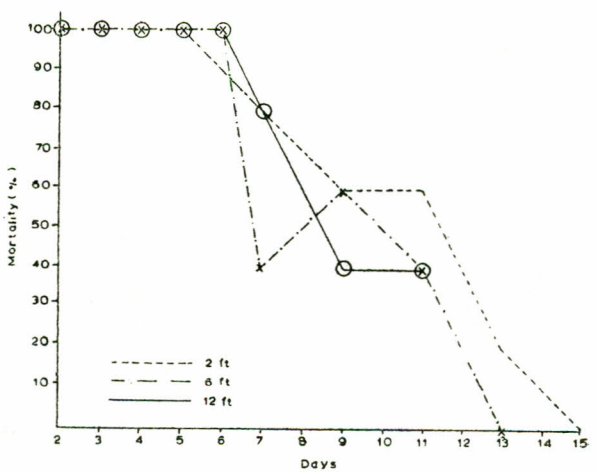

Fig. 5. Residual toxicity.

Experiment 4

Effect of the Age of Plant. Tests conducted on this aspect indicated that younger plants were more effiecient in the translocation of the insecticide than the older ones. For instance, a 50-day old plant was found to be capable of translocating sufficient Monocrotophos to provide 100% kill of pest insect larvae throughout the plant within 24 hr of application whereas 90-day old plant could not produce such results until 3 or 4 days after application (Fig. 8).

Experiment 5

Chemical Assay. In order to ascertain whether distribution of the insecticide within the plant was uniform and complete, small scale TLC experiments were conducted on plants treated with 24% granules. For these tests leaves were taken from near to base, the middle, and the tip of the plant. Each leaf was then macerated and the homogenate was extracted

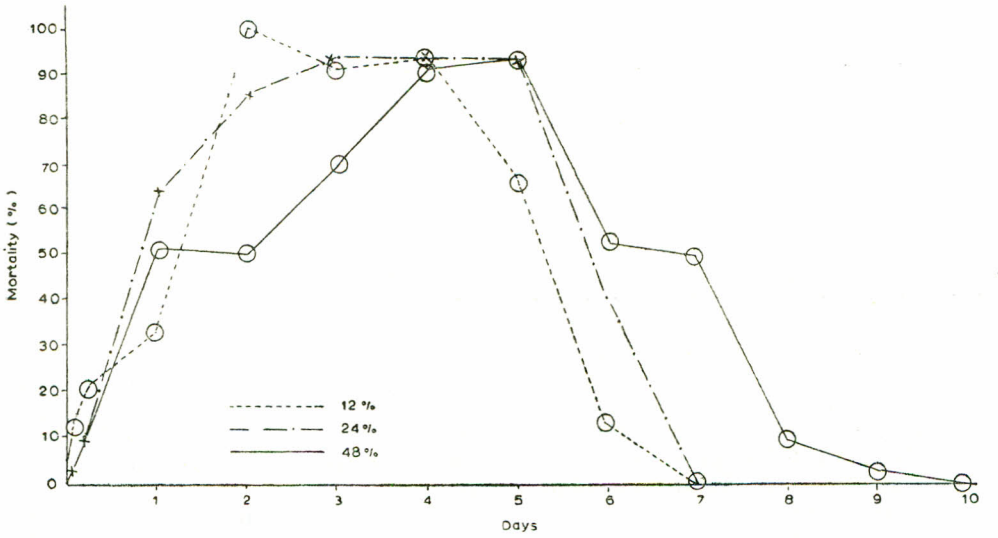

Fig. 6. Comparative effect of various concentration of granules on kill and persistance.

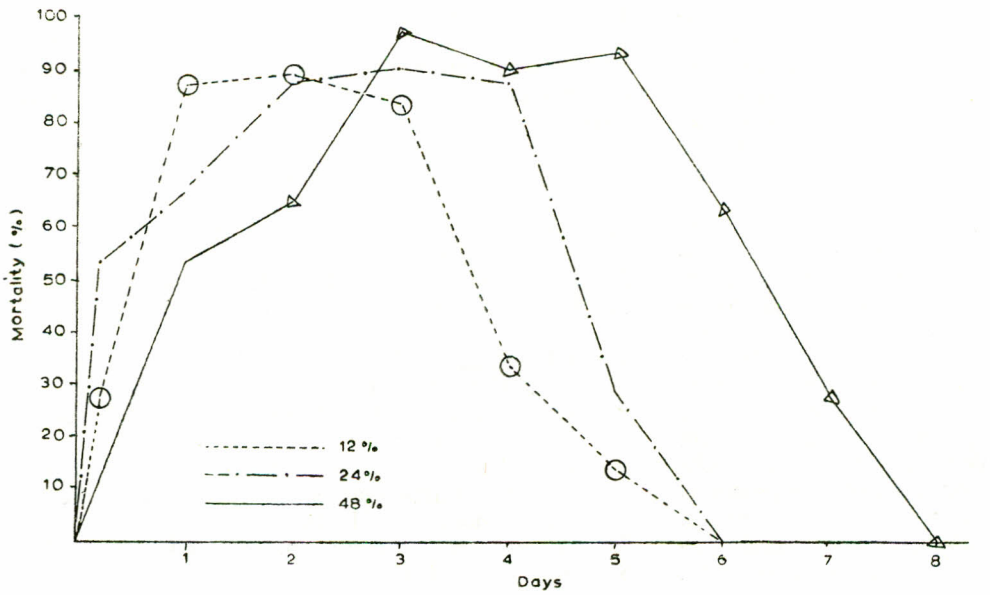

Fig. 7. Comparative effect of various concenration of granules on kill and persistance.

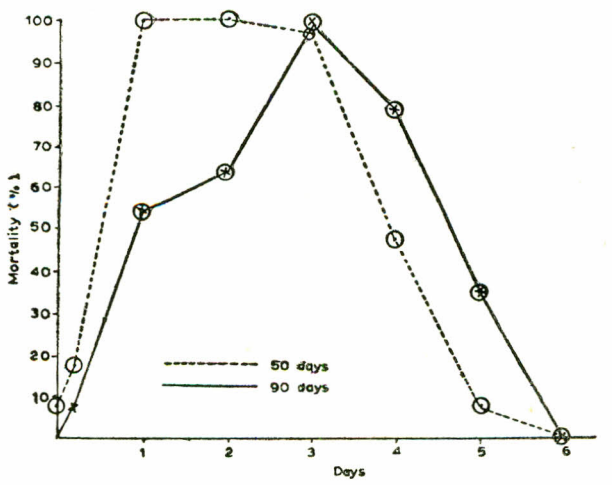

Fig. 8. Effect of age of plant on translocation.

with 50 ml chloroform and washed with (Na_2SO_4, 2%) to remove the moisture present. The extract was then filtered through a cotton plug placed in a glass funnel and was again washed with 10 ml chloroform. Ultimately the volume of the extract was reduced to about 0.5 ml on a steam bath at 80±2°C. For TLC silica-coated paper (Whatmann SG 21) was used.

The developing solution used consisted of benzene and acetone (1:1) and the chromogenic reagent comprised of 2.0% solution of 4-*p*-nitrobenzyl-*n*-pyridine in distilled acetone and 10% solution of tetraethylpentamine in distilled acetone. Two dimensional TLC of Monocrotophos standard and the plant extract was performed according to the procedure described by Gardner.[2] The *Rf* value of Monocrotophos was found to be in the range of 0.44–0.53.

Results indicated that distribution of insecticide was uniform throughout the plant (Table 1).

TABLE 1. QUANTITY OF THE INSECTICIDE (in μg/cm²) FOUND IN THE TREATED PLANTS BY TLC.

Position of leaf on the plant	Residues found after days						
	1	2	3	4	5	6	7
On lower part	0.41	0.833	1.663	0.410	0.104	0.083	Not detected
On middle part	0.41	0.410	0.833	0.274	0.166	0.083	,,
On upper part	0.50	0.538	1.070	0.352	0.216	0.108	,,

Results and Discussion

The result demonstrate that even small quantity (100 mg) of Monocrotophos granules placed inside the plant stem is translocated as far as 24 ft and provides good kill of *Epilachna* (adults and larvae) for 5 and 13 days when insects were exposed to treated plants for 24 and 48 hr respectively. Bioassay and TLC indicates that this insecticide is evenly distributed in the plant. It has been observed that in younger plants insecticide is evenly translocated within 24 hr whereas in older plants it takes 3–4 days. It has further been found that 24-hr exposure gave effective mortality up to 5 days whereas 48 hr exposure provide kill up to 11 days. It has been noticed that translocation of insecticide is comparatively earlier with 24% granules than with 48%.

These results indicate that present techniques of granule application can effectively be used for the control of foliar and stem pests infesting cucurbits including pumpkin, melon, bitter gourd and bottle gourd etc. It appears that the above described method of application would also prove successful as effective control of pests has been obtained by other workers employing stem injection techniques on elm[3] and pine trees [7,8] and by applying a band around the stem of cotton plants.[6] Convenience of application without the use of any spray or dusting equipment is the most important advantage of this technique. Moreover, insecticide so applied may remain unaffected by weathering due to rain, wind, sunlight, dew or temperature. Another benefit to be derived from the use of this method is the full utilization of the quantitity of insecticide placed inside each plant, resulting ultimately in economy on the cost of treatment and elimination of drift and its associated problems.

No tests have been conducted in the present studies to evaluate the translocation of residues of the pesticides in fruit. It is proposed to investigate this aspect at some later stage.

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