JOINT ACTION OF NUCLEAR POLYHEDROSIS VIRUS OF *Spodoptera litura* (Fabricius) WITH INSECTICIDES AND ITS APPLICABILITY IN PEST CONTROL

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DECLARATION

I hereby declare that this thesis entitled "Joint action of nuclear polyhedrosis virus of *Spodoptera litura* (Fabricius) with insecticides and its applicability in pest control" is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title of any other University or Society.

JOHN V. CHERIAN

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CERTIFICATE

Certified that this thesis entitled "Joint action of nuclear polyhedrosis virus of *Spodoptera litura* (Fabricius) with insecticides and its applicability in pest control" is a record of research work done independently by Sri. JOHN V. CHERIAN under my guidance and that it has not previously formed the basis for the award of any degree, fellowship or associateship to him.

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INTRODUCTION

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Use of insect pathogenic viruses as insecticidal agents is gaining considerable importance. Commercial products of four viruses, the Heliothis nuclear polyhedrosis virus (NPV) (Elcar), Tussockmoth NPV (TM-Biocontrol), Gypsymoth NPV (Gypcheck) and Cytoplasmic polyhedrosis virus of Dendrolimus spectabilis (Motsukimin) are available for use. Many others have been given experimental use permit. Most of these insect viruses are safe to other forms of life while they are capable of causing epizootics in populations of their specific hosts. But their high host specificity and long incubation period restrict their use in situations involving pest complexes and pest outbreaks. Under such conditions chemical insecticides are preferred. But the unrestrained use of insecticides often lead to deleterious consequences like disruption of ecosystem, development of insect resistance, pest resurgence and toxic hazards. These problems have stimulated interest in evolving a pest control strategy involving the use of pesticides in harmony with other methods of control.

The insect pathogens especially insect viruses are useful tools in an integrated approach to pest control. Before chalking out such a programme the compatibility of the candidate pathogens with insecticides has to be assessed.

Insects undergoing a stress condition like over-crowding, malnutrition and other environmental factors will be more susceptible to a disease (Steinhaus, 1958; Vago, 1959). There are reports indicating that insecticides act as stressors to viral infection (Benz, 1971). Similarly there are also reports showing viral infections weakening the tolerence of insects to insecticides (Girardeau and Mitchell, 1968). The advances made in this area of research up to 1968 were reviewed by Benz (1971). Since then a number of workers have reported the successful potentiation of viruses when mixed with low doses of insecticides (Harper and Thompson, 1970; Jaques, 1970, 1971, 1973, 1977; Atiger, 1971; Watanbe 1971, Chapman and Ignoffo, 1972; Vail et al. 1980; Yearian et al. (1980). Such combinations would help in reducing the quantity of chemical and biological agents used for pest control while accomplishing adequate crop protection.

In India, studies on the use of insect viruses in general and that on virus-insecticide combinations in particular are rather limited (Komolpith and Ramakrishnan, 1978; Chaudhari and Ramakrishnan 1980; Jayaraj 1981a, 1981b; Narayanan, 1981; Santharam et al. 1981; Savanurmath and Mathad, 1981 and Susamma Mathai, 1982).

The nuclear polyhedrosis virus (NPV) of *Spodoptera litura* (F.) was first reported in India by Pawar and Ramakrishnan (1975) and the virus was found to be effective in controlling the pest under field conditions (Santharam et al. 1978). The present study was taken up to gather information on the feasibility of combining the NPV of *S. litura* with common insecticides. The investigations carried out included bioassay of the virus on third instar larvae of *S. litura*, effect of different combinations of the virus and insecticides on larval mortality and the effect of simultaneous and sequential application of virus and insecticides for the control of the pest.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

A brief review of the literature on the joint action of insect viruses and insecticides as evaluated in the laboratory and field is given below.

Laboratory studies:

Schmidt (1959) found that nuclear polyhedrosis virus of *Lymantria dispar* was activated by sublethal doses of aldrin, dieldrin and endrin.

Kovacevic (1959) tested the activation of the nuclear-polyhedrosis virus of *Lymantria dispar* by sublethal doses (5-10 ppm) of DDT or lindane which produced 100 per cent and 70-100 per cent mortality respectively while the natural mortality reached 25-55 per cent. Aruga (1963) reported the activation of latent viroses by chemicals such as NaF, KNO_2 and H_2O_2.

Ignoffo and Montoya (1966) carried out laboratory experiments on corn earworm (*Heliothis zea*) with combinations of NPV and insecticides like DDT, endrin, carbaryl, toxaphene and methyl parathion. Carbaryl showed a slight synergistic effect while methyl parathion reduced the pathogenicity of the virus.

Vasiljevic (1967) tested three doses of the cytoplasmic polyhedrosis virus of Lymantria dispar with three toxic concentrations of DDT and a toxic and two subtoxic doses of lindane. DDT showed independent synergism whereas lindane even at toxic concentrations had no effect on the rate of mortality. Some antagonistic effect of the toxic dose, was indicated.

Girardeau and Mitchell (1968) working with Trichoplusia ni found much reduced LD_{50} values for endrin, endosulfan and trichlorfon if the larvae had been infected previously with a sub-acute dose of a nuclearpolyhedrosis virus. Komolpith (1975) found that NPV combined with pyrethrum gave the highest mortality in the larvae of Spodoptera litura.

Saad and Amin (1975) carried out laboratory tests to determine the effectiveness of Biotrol VHZ in combination with the insecticides chlorphyrifos, leptofos, methomyl, methamidofos and monocrotophos on S. littoralis. Joint action effects were observed with all combinations (except that of the virus with monocrotophos where the effect was additive) especially when the insecticides were applied 48 hours after the virus inoculation.

Komolpith and Ramakrishnan (1978) studied the joint action of NPV of Spodoptera litura and sublethal concentrations of bactospeine (B.t) and the insecticides DDT, BHC, lindane, malathion and pyrethrin. Supplemental effect was seen when the virus was combined with DDT at 5 ppm or pyrethrin at 10,20 or 50 ppm. Pyrethrin at 5 ppm when combined with the virus showed potentiating effect. Combination of virus and bacteria produced only a sub-additive effect. BHC showed an additive effect only at 50 ppm. Malathion was antagonistic to virus.

Savanurmath and Mathad (1981) studied the effect of simultaneous and sequential application of nuclearpolyhedrosis virus and fenitrothion on Mythimna separata (Wlk). Out of 51 combinations tried only nine had potentiating synergism. The most effective combinations were those of the LC_{50} of virus followed by LC_{25} of fenitrothion at seven day lag and LC_{25} of fenitrothion followed by the LC_{25} of virus one day later. The time lapse between applications in sequential combinations produced better effects and considerably reduced the quantity and cost of fenitrothion and virus compared with synergism obtained by simultaneous combination.

Savanurmath and Mathad (1982) studied the effect of simultaneous and sequential application of NPV and endosulfan on Mythimna separata (Wlk) using combinations of LC_{10}, LC_{25} and LC_{50} doses of endosulfan and the virus. Of the 51 combinations tested only 8 produced significantly high mortality. None of the simultaneous treatments of virus and endosulfan produced potentiating effect. Most of the post infection treatments of endosulfan, with 7 days lag specially in combination with high concentration virus, produced significantly high mortality showing potentiating effect. The sex ratio and male pupal weights of the surviving insects were also affected by post infection treatments of endosulfan at higher concentrations.

Shapiro and Bell (1982) tested 17 chemicals mostly inorganic and organic acids in combination with the NPV of Lymantria dispar (L) for enhanced pathogenicity. Of these two acids (Sorbic and Boric) appeared to enhance viral activity, which was expressed by increased mortality in tested larvae. Effect of boric acid was thoroughly investigated and it was found that time from inoculation to initial mortality as well as the LT_{50} were reduced by the mixing.

Susamma Mathai (1982) tested the effectiveness of insecticide-NPV combinations against S.mauritia. Synergistic effect was reported for quinalphos, fenthion and permethrin used in combination with NPV. Phosphamidon and phosalone showed synergistic effect at lower doses and antagonistic effect at higher doses while fenitrothion produced just opposite results. For endosulfan-NPV combinations the effect was synergism at higher and lower doses and antagonism at intermediate doses.

Field studies:

Genung (1960) obtained effective control of *Trichoplusia ni* by a virus-toxaphene combination and it was found far superior to either of the materials used alone. Hofmaster and Ditman (1961) found interspecific economic synergism of the virus with dibron, mevinphos, endrin, parathion, toxaphene-parathion and malathion-perthane. Getzin (1962) reported the effectiveness of combinations of NPV with insecticides for control of the cabbage looper *T. ni*. Combinations of the polyhedra with parathion or toxaphene gave significantly better control over any of the materials used singly. The larvae caused less damage to plants treated with the parathion polyhedra combinations than to those treated with either component separately, but there was little difference between the virus-toxaphene combination and toxaphene applied alone.

Wolfenbarger (1965) got good cabbage looper control when combinations of the polyhedrosis virus with DDT, endrin, mevinphos, parathion, toxaphene and DDT-toxaphene were used. He found that a virus-oil-endrin combination was significantly superior to a three fold increase in virus dosage.

Hafez et al. (1970) conducted field tests in Egypt to evaluate the effectiveness of combinations of suspensions of polyhedrosis virus and chemical insecticides in controlling larvae of Spodoptera littoralis (Boisd) on cotton. The best treatment was a combination of a mixture of endrin and dicrotophos (Bidrin) applied at the rate of 1.5 litres per feddan (0.42 ha) and the virus suspension (100 million PIBs/ml) at 24 litres.

In aerial application of virus-insecticide combinations against sprucebudworm Choristoneura fumiferana Morris et al. (1972) observed that NPV-fenitrothion mixture was the most effective in causing population reduction and foliage protection.

Jaques (1973) obtained effective control of T. ni. and Pieris rapae on cabbage by combinations of viruses with endosulfan or methomyl and suggested it as an effective method for reducing the number of applications and amount of chemical insecticides. Application of 0.25 lb of methomyl per acre combined with T. ni. NPV was as effective as 0.5 lb of methomyl per acre.

In a two year study of virus insecticide combination for the control of spruce-budworm, *Chroistoneura fumiferana* the application of NPV - fenitrothion combination was found to be highly effective (Morris et al. 1974).

Jaques (1977) tested the field efficacy of combinations of *T. ni.* NPV and methomyl at reduced rates and obtained excellent control of *T. ni.* A mixture of *Autographa californica* NPV with chlordimeform was the most effective among the treatments and the treated plots suffered least leaf damage and yield loss. Plots treated with 1/8 of the recommended dosage of chlordimeform combined with 1/5 of the normal dosage of the *Pieris rapae* granulosis virus yielded 100 per cent marketable crop being equal or superior to the yield from plots treated with the full dosage of virus or chemical.

Jaques and Laing (1978) studied the efficacy of mixtures of *Bacillus thuringiensis*, viruses and chlordimeform against cabbage pests and found that mixtures at low concentrations were as effective or more effective than the materials used alone at full rates.

McLeod et al. (1978) studied the effectiveness of microbial and chemical insecticides on beet army worm on soybean using two experimental insecticides, FMC 33297 (a synthetic pyrethroid) and UC 51762 (a carbamate), a combination of toxaphene-methylparathion-chlorpyrifos and two baculoviruses (NPV from *Autographa californica* and *Spodoptera exigua*). All treatments at 8 days post treatment significantly reduced larval numbers.

Pieters et al. (1978) evaluated the effectiveness of mixtures of chlordimeform and Elcar against *H. zea* and *H. virescens* on cotton. The mixture compared favourably with conventional chemical insecticides (Chlorodimeform alone or mixed with monocrotophos or toxaphene and methyl-parathion alone or mixed with EPN). The results showed that chlordimeform did little to increase the effectiveness of the virus preparation.

Luttrell et al. (1979) combined permethrin, methomyl and EPN-methyl parathion with Elcar in laboratory and field studies. No significant differences were detected between observed and expected mortality of *H. zea* larvae in the laboratory although slight increases in mortality were observed in some tests. In a small plot test on cotton, reduced rates (0.125, 0.25 and 0.5 x the recommended rate) of permethrin, methomyl and EPN-methyl parathion were combined with a recommended rate of Elcar. Permethrin-Elcar and methomyl-Elcar plots produced yields equal to those treated with Elcar alone, while EPN-methylparathion plots had reduced yields.

Choudhari and Ramakrishnan (1980) determined the effectiveness of the NPV of Spodoptera litura in combination with sublethal doses of DDT and endosulfan against the pest artificially infested on cauliflower. The extent of damage to the cauliflower leaves was reduced in the case of leaves sprayed with virus in combination with DDT (31.2 per cent foliage damage). Combination of virus with 50 ppm endosulfan did not increase foliar protection over that obtained with virus alone (43.7 per cent foliage damage)

Santharam and Balasubramonian (1980) conducted field tests using combinations of NPV and insecticides like chlorpyrifos and dichlorvos and diflubenzuron on tobacco against Spodoptera litura. Effective control of the pest was obtained when 0.04 per cent of chlorpyrifos and dichlorvos were used in combination with NPV @ 125 LE/ha. NPV at doses 15×10^{11} PIBs/ml (250LE), 7.5×10^{11}PIBs/ml (125LE) and diflubenzuron gave only less than 30 per cent control 48 hours after application but it gave good control after one week.

Vail et al. (1980) reported that combinations of the A. californica NPV with reduced doses of chemical insecticides provided good control as chemicals alone against

T. ni damage to lettuce heads.

Yearian et al. (1980) evaluated the effectiveness of mixtures of various formulations of *Bacillus thuringiensis* or *Baculovirus heliothis* with chlordimeform for the control of larvae of *Heliothis* spp. on cotton in a series of small plot tests. Treatment with mixtures of *Baculovirus heliothis* and chlordimeform generally gave viable results, but certain combinations particularly those including gustatory adjuvants produced yields that were significantly higher than those resulting from treatments with either of the materials alone.

Jayaraj (1981a) reported the effectiveness of NPV against *Heliothis armigera* on chickpea under field conditions. The virus at 125 LE/ha along with endosulfan at half the recommended dose (0.035 per cent) was significantly effective in reducing the pest damage to both pods and grains.

Field trials conducted on Varalakshmi hybrid cotton against *S. litura* (F) showed that the virus at 50 LE combined with half the recommended dose of chlorpyrifos at 0.51/ha caused 52.4 per cent mortality which was on par with mortality caused by chlorpyrifos alone at 1 l/ha level. It was also noticed that application of dichlorvos and methamidophos at concentrations 0.01 per cent and 0.5 l/ acre with reduced dosage of NPV resulted in 72 per cent mortality of larvae (Jayaraj, 1981b).

Narayanan (1981) observed that when a combination of NPV at 125 LE/ha and endosulfan 0.035 per cent was used against podborer (*Adisura atkinsoni*) on field beans, the total pod damage was reduced to 25.83 per cent as compared to endosulfan 31.6 per cent in endosulfan treated plots and 38.53 per cent in virus treated plots.

Santharam et al. (1981) found that NPV at 375 LE/ha recorded 71.67 per cent mortality and was on par with combination sprays of monocrotophos 0.02 per cent plus NPV at 125 LE/ha and endosulfan 0.035 per cent with NPV at 125 LE/ha for the control of *H. armigera* on redgram.

Bell and Romine (1982) reported that treatment with a mixture of the NPV of *Autographa californica*, *B. thuringiensis* and feeding adjuvant on a five day schedule resulted in the highest control of cotton leaf perforator.

MATERIALS AND METHODS

MATERIALS AND METHODS

Mass rearing of caterpillars of *Spodoptera litura*

The larvae of *S. litura* used in these studies were obtained from an aseptic culture maintained in the laboratory. The culture was initiated from a single egg mass collected from the field.

The eggs were surface-sterilized by immersing them in 10 per cent formalin for one hour following the method of Henneberry and Kishaba (1966). They were washed several times in sterile distilled water and were then air dried. Those eggs were transferred to clean sterilized glass troughs and tender leaves of castor (*Ricinus communis*) with stalks dipped in water in specimen tubes were placed in the troughs. The troughs were covered with muslin cloths. On hatching, the larvae migrated to the tender leaves which served as their food. The second instar larvae were transferred to sterilized glass troughs in batches of 25-30 per trough and fresh castor leaves were provided as food everyday. Larvae showing symptoms of bacterial or other infections were removed promptly. The full grown larvae were transferred to glass troughs containing 3 cm layer of clean sterilized sand for pupation.

Adult moths on emergence were kept in glass troughs in batches of 4 to 5 pairs for egg laying. Cotton swabs soaked in ten per cent honey solution was provided to feed the moths. Castor leaves with stalks dipped in water taken in specimen tubes were kept in jars to serve as substrates for egg laying. Egg laying commenced on the third day after emergence and continued for 5 to 6 days. The egg masses were collected daily, sterilized and kept for hatching as described earlier.

Contamination of larvae was minimised by maintaining high sanitation in rearing. Glasswares were sterilized by immersing them in 0.5 per cent sodium hypochlorite solution for one day (Wittig, 1963). They were then washed in sterilized distilled water and air dried. Smaller glasswares like petridishes, tubes etc. were sterilized in a hot air oven at 180°C for three hours. The food plants were washed thoroughly in running sterile water prior to feeding. Crowding of larvae in individual trough was avoided.

Preparation and storage of polyhedral inoculum

The primary inoculum was obtained from a purified polyhedral suspension of the NPV of *S. litura* preserved at the Division of Agricultural Entomology, College of

Agriculture, Vellayani. It was multiplied by feeding third instar larvae of S. litura on semitender castor leaves contaminated with the inoculum. The dead larvae were collected and allowed to putrify in sterile distilled water in conical flasks for 2 to 3 weeks (Smith 1967). The polyhedra which settled as a thin white layer at the bottom were collected and purified by filtration and centrifugation. When the putrification was incomplete the polyhedra were extracted by maceration of dead larvae in a warring blender and purified by centrifugation. The purified polyhedra were resuspended in sterile distilled water and stored in a refrigerator at $4^{o}C$.

Concentration of the stock suspension was estimated to be 29×10^{9} PIBs/ml by taking counts of polyhedra using a Neubauer improved double ruled haemocytometer as described by Lewis (1960). Serial dilutions required for the various experiments were prepared from this stock suspension using sterile distilled water.

Inoculation of caterpillars with the virus

Semitender castor leaves were used as the feeding material. Purified polyhedral suspension containing 0.1 per cent teepol as wetting agent was used as the inoculum. The leaves were sprayed on both sides with inoculum using a Potter's tower at 2.5 kg/cm^2 pressure. The cut end of the leafstalk was covered with a wet cotton swab so as to keep the leaves turgid. The leaves were then air-dried at room temperature. Circular leaf discs having area of 5.3 cm^2 were cut from the treated leaves and each leaf disc was introduced into a specimen tube. One larva was released in each tube and the open end of the tube was covered with muslin cloth and secured with rubber band. The larvae were forced to starve for 6 hours, before release on the treated leaves. The larvae which had completely consumed the treated leaves in 12 hours were transferred to fresh castor leaves in specimen tubes. This method ensured the feeding of a more or less uniform area of leaves, treated with the virus, by all larvae. Larvae fed similarly on castor leaf discs treated with 0.1 per cent teepol alone served as control.

Bioassay of the NPV.

The test larvae were drawn from the disease free laboratory stock of *S. litura*. Freshly moulted third instar larvae of uniform size were used in the bioassay studies.

Five serial dilutions of the virus, viz., 29×10^6, 14.5×10^6, 7.25×10^6, 3.62×10^6 and 1.81×10^6 PIBs/ml were prepared from the stock suspension. These concentrations were fixed by preliminary exploratory tests, so that mortalities ranging from 20 to 90 per cent were obtained. The larvae were inoculated with the virus adopting the technique described earlier. Selection of leaf discs of uniform size and exclusion of larvae which did not completely consume treated leafdiscs in 12 hours ensured a uniform dosing of the test insect. The larvae fed on treated leaves were transferred to sterile specimen tubes individually, and were supplied with fresh leaves. Larvae of the same age and size treated with teepool alone were maintained as control. The experiment was replicated thrice with ten larvae in each replication. The larval mortality till pupation of survivals was recorded. In doubtful cases, mortality caused by nuclear-polyhedrosis virus was confirmed by microscopic examination of smears. The data obtained were subjected to probit analysis (Finney, 1952). LC_{50} value of the virus for the third instar larvae was calculated.

Bioassay of insecticides.

Bioassay of quinalphos, carbaryl, BHC, monocrotophos and permethrin was done in the laboratory for assessing their relative efficacy against third instar larvae of *S.litura*.

Five graded concentrations of each insecticide were used. The doses were so spaced to ensure mortalities ranging from 20 to 90 per cent. For this, preliminary explorative trials were conducted. The graded concentrations of the insecticides were prepared by diluting the emulsifiable concentrate/wettable powder with water. Third instar larvae of S. litura were selected and starved for six hours prior to the treatment. Semitender leaves of castor were treated with one ml of each of the insecticide emulsion/suspension under a Potter's tower and the treated leaves were air dried. Circular leaf discs (5.3 cm^2) cut from treated leaves were put in specimen tubes. One caterpillar was confined in each specimen tube using cotton plugs. After 12 hours of feeding the caterpillars were removed to clean tubes individually and fed with fresh leaves. Mortality counts were taken upto 72 hours after exposing the larvae to the treated leaves. Each treatment was replicated thrice and ten larvae were used in each replication.

The dosage-mortality data were subjected to probit analysis (Finney, 1952) and the regression equations and LC_{50} values were worked out.

Bioassay of NPV-insecticide mixtures.

The median lethal dose of the virus was combined with five graded doses of the insecticides, viz., LC_{10}, LC_{20}, LC_{30}, LC_{40} and LC_{50}. These doses of insecticides were computed from the regression equations derived by probit analysis of logdose-mortality data.

The bioassay technique used here was the same as that used for insecticides alone. *S.litura* larvae which had completely consumed the leaf discs treated with virus-insecticide mixture in 12 hours were separated out and fed with fresh leaves and reared individually in specimen tubes. Each treatment was replicated thrice with ten larvae in each replication. Observations were recorded on larval mortality at 24 hour intervals and were continued till pupation of survivals. The final mortalities were compared using test of significance of proportions (Nageswara Rao, 1983).

Categorisation of joint actions between NPV and insecticides

The joint actions of the virus and the insecticides were grouped under different categories based on the criteria proposed by Benz (1971).

Independent synergism (Independent action with zero correlation)

This was calculated using the formula

$M(v + i) = M_v + M_i \left(1 - \frac{M_v}{100}\right)$ where, M_v was per cent mortality caused by the virus alone, M_i the per cent mortality caused by insecticide alone and $M(v + i)$ was the probability of death by the combined action of the virus and insecticide.

Sub-additive synergism

If the virus-insecticide mixture gave mortality greater than independent synergism but less than the algebraic sum of the two single effects, it was classified as sub-additive synergism. A weak potentiating effect was considered necessary to produce such a result.

Supplemental synergism

If the virus-insecticide mixture gave a mortality greater than the algebraic sum of the single effects it was classified under supplemental synergism, ie.

$$M(v + i) > M_v + M_i$$

Potentiating synergism

If by mixing a particular dose of the virus giving a particular percentage of mortality with a nonlethal or sublethal dose of an insecticide, the mixture gave a substantially higher mortality than that given by the virus alone it was classified as potentiating synergism, ie. if the combined mortality was significantly higher than the calculated independent action with zero correlation (Savanurmath and Mathad, 1982).

Antagonism

When NPV was combined with an insecticide the mortality given by the mixture was less than the probable mortality caused by the combined action of the two components acting independently, it was categorised as antagonism.

Use of NPV-insecticide mixtures for the control of *S. litura*.

Simultaneous application of NPV and insecticides.

Cowpea plants were raised in pots (30 x 30 cms). When the plants were three weeks old fifteen third instar larvae of *S. litura* were released in each pot.

Combinations of virus and insecticides found promising in the laboratory experiments were used for field trial.

Quinalphos at LC_{10} and LC_{50} and permethrin at LC_{30} and LC_{40} levels in combination with median lethal dose of virus were the treatments chosen. The insecticides and virus were included independently in the treatments. Thus there were six treatments and each treatment was replicated thrice. The spraying was done with an atomizer. A uniform quantity of 50 ml of the spray fluid was applied on the plants in each pot. Plants sprayed with water alone were kept as control. The pots were covered with polythene cages to confine the larvae to the plants. The larvae were allowed to feed on the treated plants for a period of 48 hours. Afterwards they were collected and reared singly in specimen tubes provided with fresh cowpea leaves as food. Daily observations were continued till the caterpillars died or pupated as the case may be.

Sequential application of NPV and insecticides

Cowpea plants were raised in pots as in the previous experiment. Fifteen third instar larvae of *S. litura* were released in each pot. The NPV-insecticides combinations tried were the same as in the previous experiment. At first 50 ml of the selected doses of the insecticides, quinalphos and permethrin were sprayed on the potted plants infested by *S. litura* larvae. After 48 hours the NPV suspension at median lethal concentration was sprayed on these plants. An equal number of larvae on untreated plants served as control. The larvae were collected from the plants 48 hours after the virus application and reared individually in the laboratory for further observation on larval mortality. The experiment was conducted in randomised block design, each treatment replicated thrice and with 15 larvae in each replication. Mortality was recorded daily till all the caterpillars died or pupated.

In another experiment cowpea plants in pots were first sprayed with an NPV suspension at LC_{50} level and after a lag period of 48 hours the selected doses of insecticides were applied. The larvae were collected from the plants 48 hours after the application of insecticides and the surviving ones were reared individually in laboratory for further observations as in the previous experiment.

RESULTS

RESULTS

LC_{50} of the nuclearpolyhedrosis virus and various insecticides to the caterpillars of *Spodoptera litura*

The results of bioassay of the virus and insecticides on third instar larvae of *S. litura* are presented in Table 1. LC_{50} of the NPV was found to be 8.62×10^6 PIBs/ml. LC_{50} values of quinalphos, carbaryl, BHC, monocrotophos and permethrin were 0.008415, 0.1709, 0.07987, 0.04920 and 0.002728 per cent respectively. Among the insecticides permethrin with LC_{50} value of 0.002728 per cent was found to be most toxic to the larvae.

Joint action of NPV and quinalphos on caterpillars of *S.litura*.

The effect of combinations of NPV and sublethal concentrations of quinalphos on the caterpillars of *S.litura* is presented in Table 2 and illustrated in Fig.1. It can be seen that the NPV insecticide combinations gave mortalities of the caterpillars from the first day onwards. The highest mortality of 96.0 per cent was recorded in the combination of LC_{50} of virus and LC_{50} of quinalphos.

Table 1. LC_{50} of the NPV and different insecticides to third instar larvae of *S. litura*.

NPV/ insecticide	Heterogeneity $\chi^2_{(3)}$	Regression equation *	LC_{50}	Fiducial limits
NPV	1.11	$y = 1.283x + 2.517$	8.62×10^6 PIBs/ml	5.44×10^6 14.31×10^6
Quinalphos	6.513	$y = 2.971x + 2.251$	0.008415%	0.006797 0.01027
Carbaryl	3.986	$y = 4.604x - 5.279$	0.1709 "	0.1535 0.1926
BHC	3.582	$y = 3.894x - 2.408$	0.07987 "	0.0674 0.0919
Monocrotophos	2.982	$y = 3.056x - 0.171$	0.04920 "	0.04143 0.05966
Permethrin	3.870	$y = 3.501x - 0.027$	0.002728 "	0.002318 0.003231

* Regression equation of probit-mortality (y) on log concentration (x)

Table 2. Mortality of third instar caterpillars of <u>S. litura</u> caused by sublethal doses of quinalphos and NPV (LC_{50}) combinations.

Treatments		Per cent mortality at the end of (days)										
NPV (PIBs/ml)	Quinal phos (%)	1	2	3	4	5	6	7	8	9	Total*	MTLD (days)
8.62×10^6	0.003 (LC_{10})	3.33	3.33	6.66	13.32	29.97	56.61	76.59	83.25	83.25	82.0	5.8
"	0.004 (LC_{20})	6.66	9.99	13.32	16.65	19.98	36.63	46.62	63.27	76.59	75.0	6.2
"	0.0056 (LC_{30})	9.99	19.98	39.96	43.29	53.28	63.27	76.59	79.92	83.25	82.0	4.4
"	0.007 (LC_{40})	19.98	26.64	43.29	56.61	66.6	69.93	83.25	86.58	86.58	85.0	3.6
"	0.0084 (LC_{50})	19.98	26.64	36.63	53.28	59.94	73.26	76.59	79.92	96.67	96.0	4.6
"	-	-	-	-	6.66	9.99	19.98	29.97	46.62	56.61	55.1	7.0

* Corrected for control mortality using Abbot's formula.

Fig. 1 Mortality of *S. litura* caterpillars caused by combinations NPV and quinalphos.

Fig. 2 Effect of combinations of NPV and quinalphos on *S. litura* caterpillars.

A - NPV LC_{50} (8.62 x 10^6 PIBs/ml) + Quinalphos LC_{10} (0.003%)

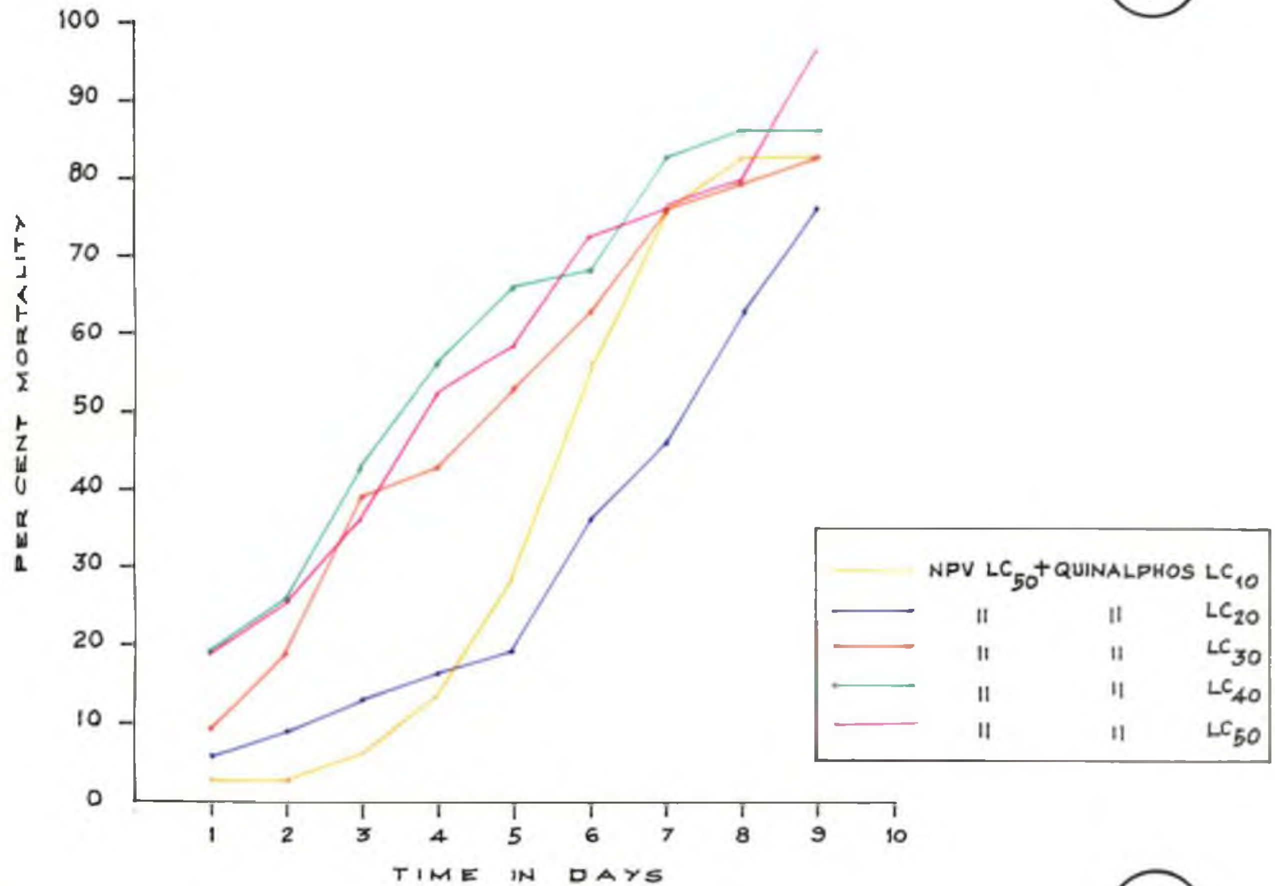
B - " " LC_{20} (0.004%)

C - " " LC_{30} (0.0056%)

D - " " LC_{40} (0.007%)

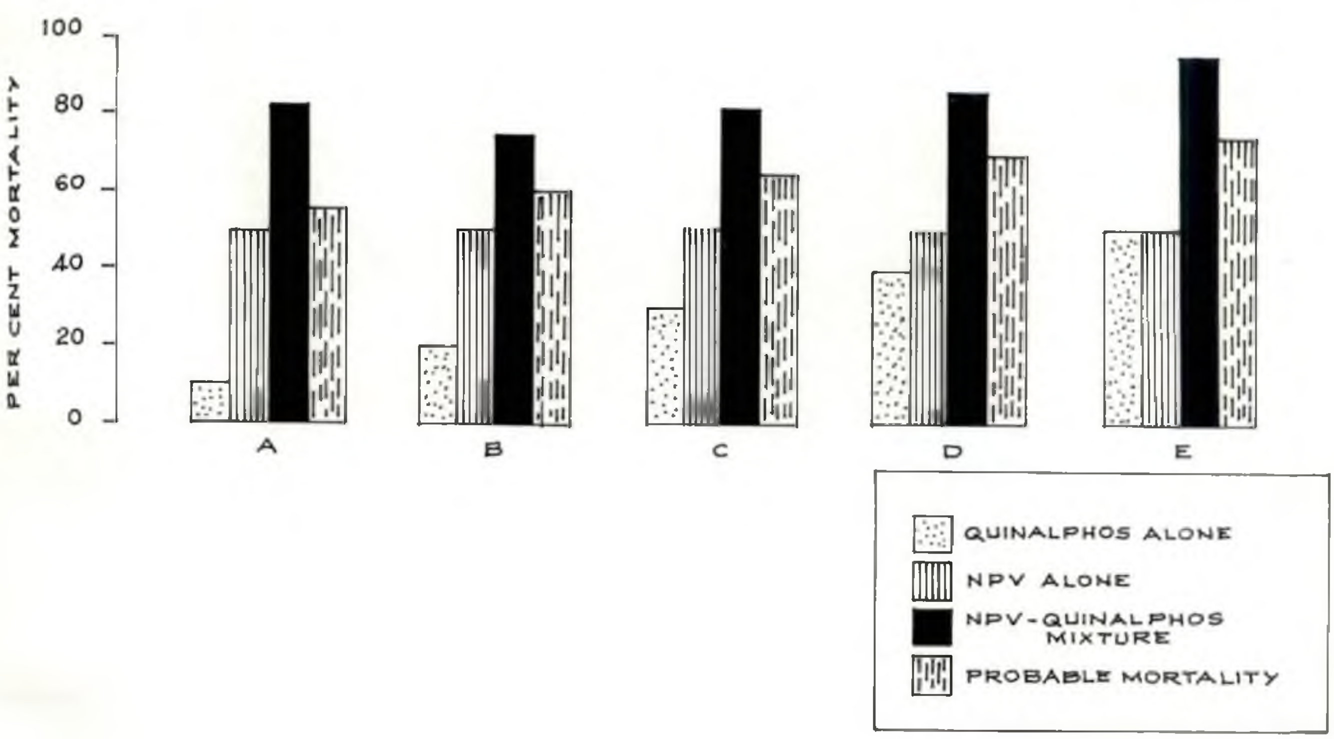
E - " " LC_{50} (0.0084%)

FIG. 1



PER CENT MORTALITY
100
90
80
70
60
50
40
30
20
10
0
1
2
3
4
5
6
7
8
9
10
TIME IN DAYS
NPV LC50 + QUINALPHOS LC10
" " LC20
" " LC30
" " LC40
" " LC50

FIG. 2



PER CENT MORTALITY
100
80
60
40
20
0
A
B
C
D
E
QUINALPHOS ALONE
NPV ALONE
NPV-QUINALPHOS MIXTURE
PROBABLE MORTALITY

But it was not significantly different from other treatments except the combination between virus and LC_{20} quinalphos. There was a steady increase in mortality of caterpillars from the third day onwards indicating that virus became active in the caterpillars during the period. Mean time for larval death (MTLD) for the combined treatments ranged from 3.6 days to 6.2 days as against an MTLD of 7 days when LC_{50} of the virus alone was applied.

The joint action of NPV and quinalphos is presented in Table 3 and Fig. 2. Combinations of all doses of the insecticide and NPV showed synergism. Combinations of the virus with LC_{10} of quinalphos caused 82 per cent mortality showing potentiating synergism. Combinations involving LC_{20} and LC_{30} of the insecticide showed supplemental synergism. But at the higher doses of quinalphos only a subadditive synergistic effect was noticed.

Joint action of NPV and permethrin on caterpillars of *S. litura*.

Data on the joint action of NPV and doses of permethrin as compared to their individual actions on the caterpillars of *S. litura* are presented in Table 4 and Fig.3.

Table 3. Effect of the combinations of NPV and quinalphos on *S. litura* caterpillars.

NPV		Quinalphos		NPV- Quinalphos mixture mortality	Probable mortality by combined action.	Effect
Concentration (PIBs/ml)	Mortality (%)	Concentration (%)	Mortality (%)	(%)	(%)	
8.62×10^6	50	0.003	10	82	55	Potentiating
"	"	0.004	20	75	60	Supplemental
"	"	0.0056	30	82	65	Supplemental
"	"	0.007	40	85	70	Subadditive
"	"	0.0084	50	96	75	Subadditive

Table 4. Mortality of *S. litura* caterpillars caused by sublethal doses of permethrin and NPV (LC_{50}) combinations.

Treatments		Per cent mortality at the end of (days)										MTLD (Days)
NPV (PIBs/ml)	Permethrin (%)	1	2	3	4	5	6	7	8	9	Total *	
8.62×10^{6}	0.0012 (LC_{10})	3.3	3.3	9.9	9.9	23.3	53.3	73.3	73.3	73.3	71.0	5.6
"	0.0016 (LC_{20})	3.3	13.3	16.7	20.0	26.6	33.3	53.3	56.6	70.0	68.0	5.8
"	0.0019 (LC_{30})	13.3	23.3	30.0	36.6	43.3	53.3	63.3	79.9	96.6	96.0	5.4
"	0.0023 (LC_{40})	20.0	23.3	23.3	33.3	46.6	66.6	79.9	83.3	93.3	93.0	5.0
"	0.0027 (LC_{50})	26.6	30.0	36.6	56.6	73.3	83.3	86.3	96.6	96.6	96.0	3.9

* Corrected for control mortality using Abbot's formula.

Fig.3 Mortality of *S. litura* caterpillars caused by combinations of NPV and permethrin.

Fig.4 Effect of combinations of NPV and permethrin on *S. litura* caterpillars.

A - NPV LC_{50} (8.62×10^6 PIBs/ml) + Permethrin LC_{10} (00012%)

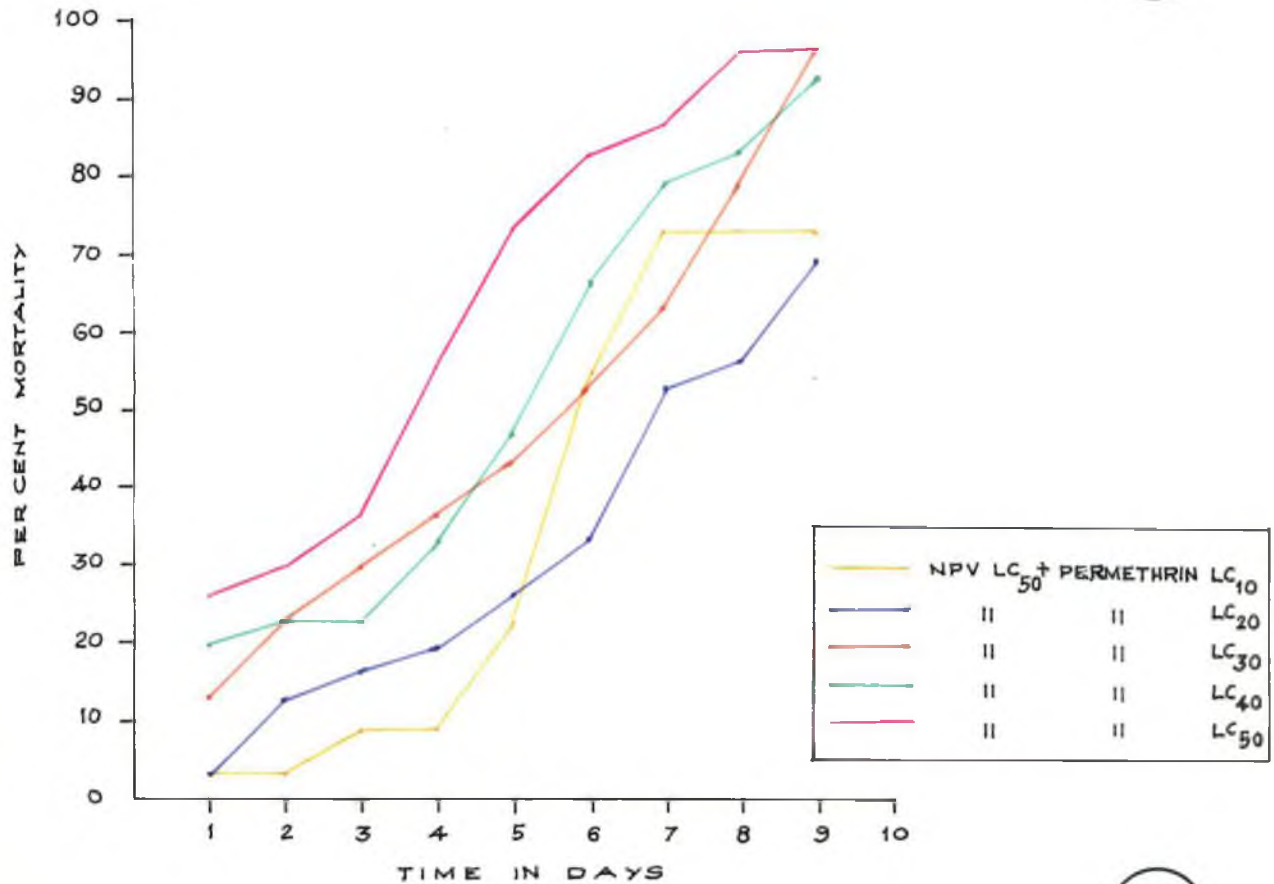
B- " " LC_{20} (0.0016%)

C - " " LC_{30} (0.0019%)

D - " " LC_{40} (0.0023%)

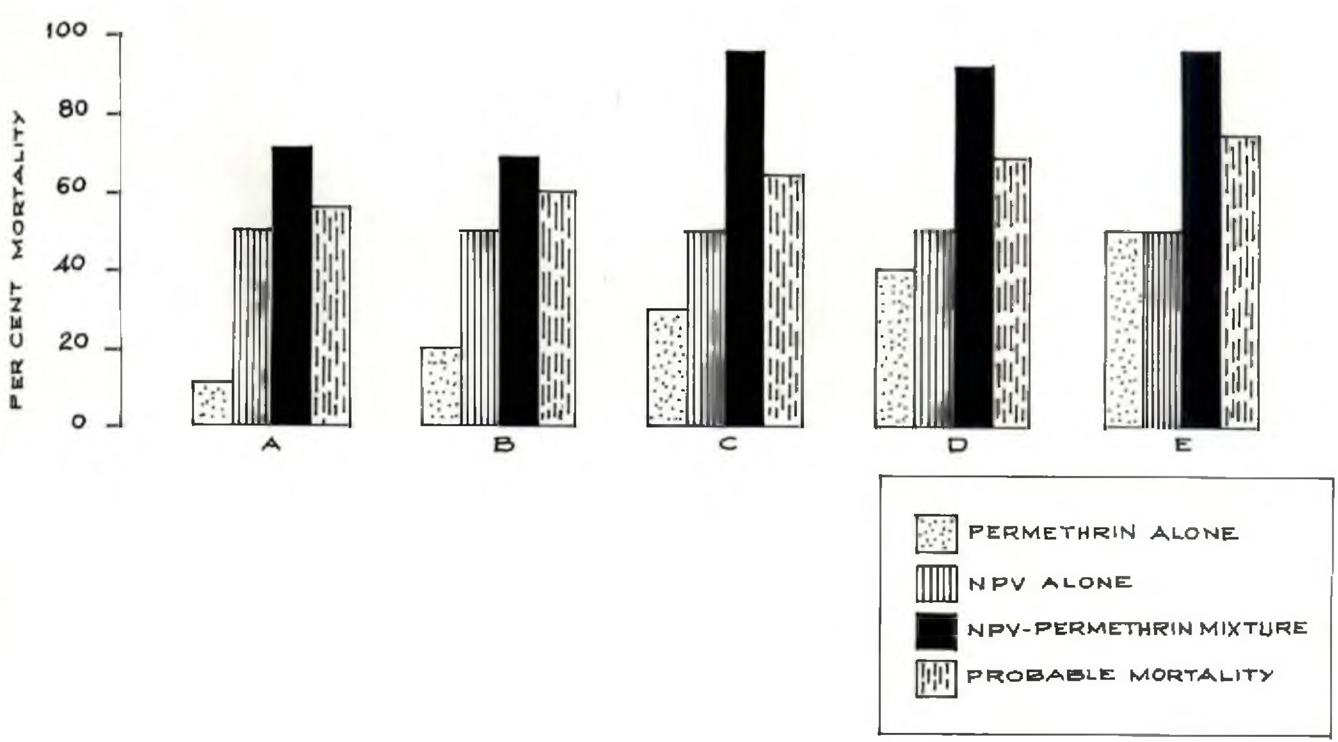
E - " " LC_{50} (0.0027%)

FIG. 3



100
90
80
70
60
50
40
30
20
10
0
PER CENT MORTALITY
1 2 3 4 5 6 7 8 9 10
TIME IN DAYS
NPV LC50 + PERMETHRIN LC10
" " LC20
" " LC30
" " LC40
" " LC50

FIG. 4



100
80
60
40
20
0
PER CENT MORTALITY
A
B
C
D
E
PERMETHRIN ALONE
NPV ALONE
NPV-PERMETHRIN MIXTURE
PROBABLE MORTALITY

Mortality of caterpillars treated with the virus-insecticide mixtures was observed from the first day of treatment. A gross correspondence between the graded doses of the insecticide-virus mixtures and per cent mortalities caused by them is evident from the results. A steady increase in the mortalities of the caterpillars under all the combinations especially from the fourth day of application was noticed indicating the activity of virus in the caterpillars. No significant difference was observed between the mortalities recorded in the treatments involving LC_{30}, LC_{40} and LC_{50} of the insecticide. The lowest MTLD of 3.9 days was noticed when LC_{50} doses of virus and insecticide were combined.

The effects of combining NPV with permethrin together with the categorisation of their combined effects are presented in Table 5 and shown in Fig. 4. It will be observed that the effect of combining NPV and sublethal doses of permethrin gave synergism ranging from subadditive to potentiating effects. In the case of combinations involving LC_{50} of the virus with LC_{30} and LC_{40} of permethrin actual mortalities of 96 and 93 per cent were observed as against their probable mortalities of 65 and 70 per cent indicating potentiating synergism between the insecti-

Table 5. Effect of the combinations of NPV and permethrin on *S. litura* caterpillars.

NPV		Permethrin		NPV-Permethrin mixture mortality (%)	Probable mortality by combined action. (%)	Effect
Concentration (PIBs/ml)	Mortality (%)	Concentration (%)	Mortality (%)			
8.62×10^6	50	0.0012	10	71	55	Supplemental
"	"	0.0016	20	68	60	Subadditive
"	"	0.0019	30	96	65	Potentiating
"	"	0.0023	40	93	70	Potentiating
"	"	0.0027	50	96	75	Subadditive

cide and virus. When LC_{50} dose of the virus was combined with LC_{10} of permethrin supplemental synergism was observed. While the combination involving LC_{20} of the insecticide gave only subadditive effect. Though the combination of LC_{50} doses of virus and insecticide recorded the highest mortality of 96 per cent it gave only a subadditive effect as the observed mortality was less than the added effects of the individual components.

Joint action of NPV and BHC on caterpillars of *S. litura*.

The data on mortality of caterpillars caused by the combinations of NPV and sublethal concentrations of BHC, in comparison with the effects of NPV at LC_{50} and BHC at sublethal concentrations are presented in Table 6 and illustrated in Fig. 5. All the virus-insecticides mixtures gave mortalities varying from 3.3 to 23.3 per cent on the first day of application. The final mortality ranged from 53.30 to 76.60 per cent in the various treatments, the treatment 8.62 x 10^6 PIBs/ml plus LC_{20} BHC recording the highest mortality. But there was a decrease in total mortality when the dosage of insecticide was increased beyond LC_{20} value indicating an antagonising effect of the insecticide at higher levels. However, there was no significant differente between treatments. MTLD ranged

Table 6. Mortality of *S. litura* caterpillars caused by sublethal doses of BHC and NPV (LC_{50}) combinations.

Treatments		Per cent mortality at the end of (days)										
NPV (PIBs/ml)	BHC (%)	1	2	3	4	5	6	7	8	9	Total*	MTLD (Days)
8.62×10^6 (LC_{50})	0.037 (LC_{10})	3.3	6.6	6.6	13.3	36.6	63.3	63.3	66.6	73.3	73.3	5.5
"	0.048 (LC_{20})	3.3	6.6	6.6	6.6	20.0	40.0	50.0	63.3	76.6	76.6	5.7
"	0.058 (LC_{30})	16.6	16.6	16.6	16.6	26.6	36.6	46.6	53.3	63.3	63.3	4.1
"	0.068 (LC_{40})	23.3	33.3	33.3	36.6	53.3	53.3	53.3	53.3	53.3	53.3	2.62
"	0.079 (LC_{50})	23.3	33.3	36.6	43.3	46.6	60.0	60.0	60.0	60.0	60.0	2.94

* No mortality was observed in control

Fig. 5 Mortality of *S. litura* caterpillars caused by combinations of NPV and BHC.

Fig.6 Effect of combinations of NPV and BHC on *S. litura* caterpillars.

A - NPV LC_{50} (8.62 x 10^6 PIBs/ml) + BHC LC_{10} (0.037%)

B - " " LC_{20} (0.048%)

C - " " LC_{30} (0.058%)

D - " " LC_{40} (0.068%)

E - " " LC_{50} (0.079%)

FIG. 5

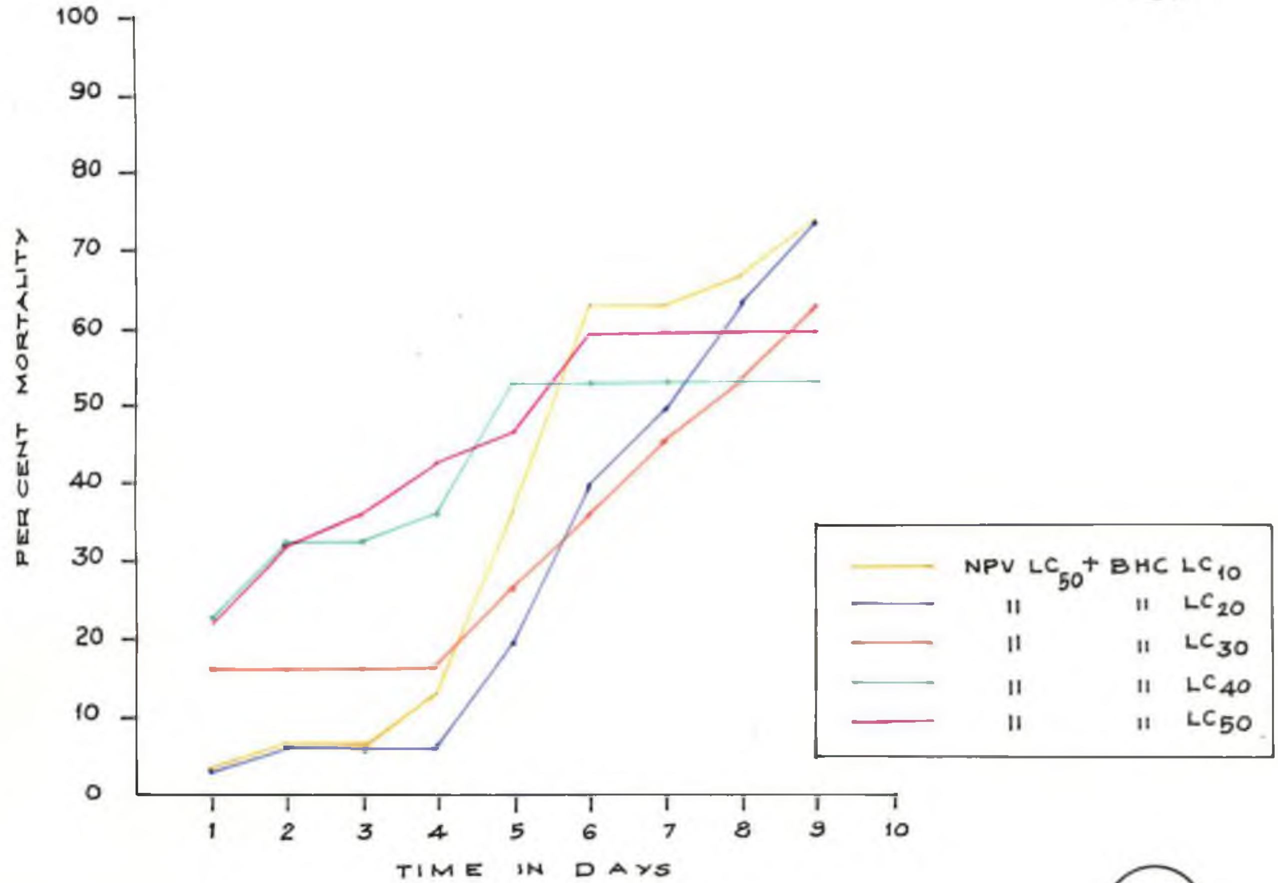
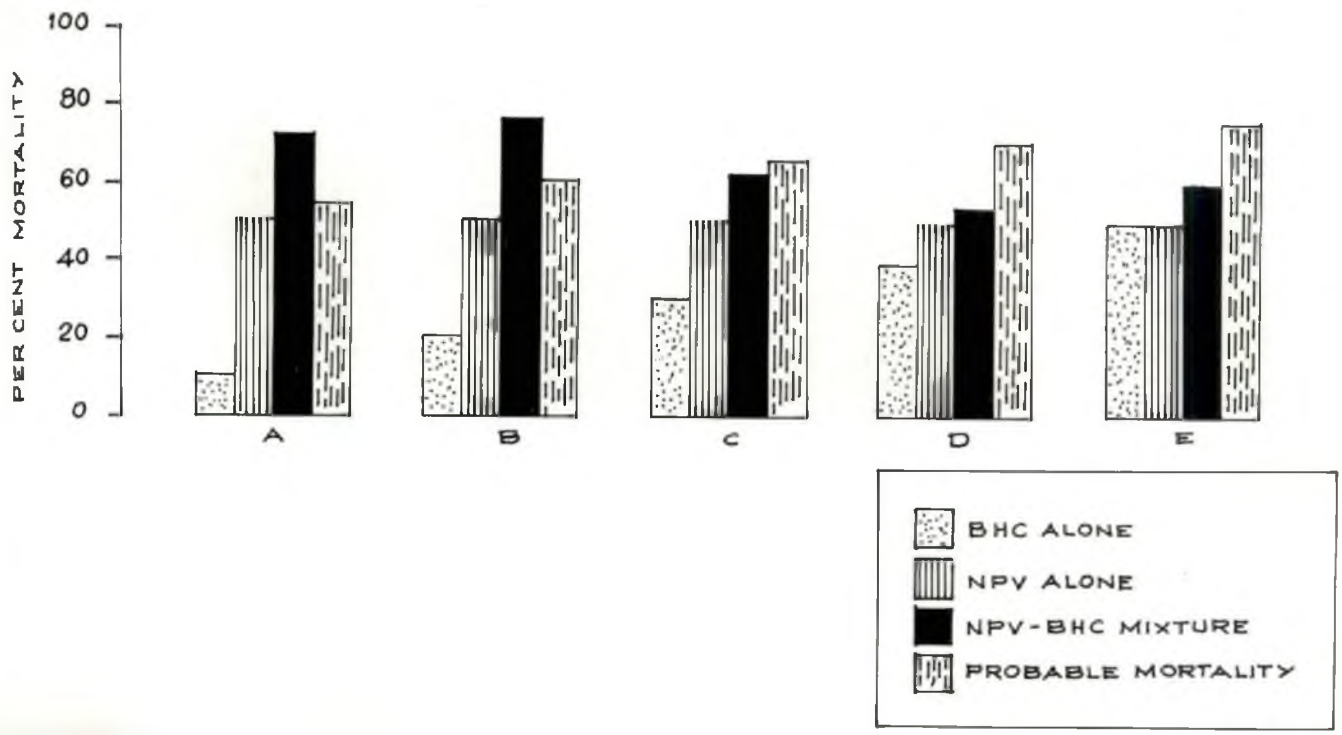

100
90
80
70
60
50
40
30
20
10
0
PER CENT MORTALITY
1
2
3
4
5
6
7
8
9
10
TIME IN DAYS
NPV LC50 + BHC LC10
" " LC20
" " LC30
" " LC40
" " LC50

FIG. 6


100
80
60
40
20
0
PER CENT MORTALITY
A
B
C
D
E
BHC ALONE
NPV ALONE
NPV-BHC MIXTURE
PROBABLE MORTALITY

from 2.62 days to 5.7 days.

The categorisation of the synergistic effect when NPV (LC_{50}) and sublethal doses of BHC are combined, is presented in Table 7. and Fig. 6. It is seen that the insecticide doses LC_{10} and LC_{20} when combined with LC_{50} dose of the virus recorded 73.3 and 76.6 per cent mortality while the expected probable mortality for these combinations were 55 and 60 per cent respectively. The effect noticed in these combinations was supplemental synergism. At higher doses of the insecticide viz., LC_{30}, LC_{40} and LC_{50} the joint effect manifested was antagonism. The above three doses of BHC in combination with the virus recorded 63.3, 53.3 and 60.0 per cent mortalities as against computed probable mortalities of 65, 70 and 75 per cent indicating an antagonistic effect.

Joint action of NPV and carbaryl on caterpillars of *S. litura*.

The mortality of caterpillars of *S. litura* caused by the various combinations of NPV (LC_{50}) and sublethal doses of carbaryl as compared to mortality due to their individual effects are presented in Table 8 and illustrated in Fig. 7.

Table 7. Effect of the combinations of NPV and BHC on S. litura caterpillars.

NPV		BHC		NPV-BHC mixture mortality	Probable mortality by combined action.	Effect
Concentration (PIBs/ml)	Mortality (%)	Concentration (%)	Mortality (%)	(%)	(%)	
8.62×10^6	50	0.037	10	73.3	55	Supplemental
"	"	0.048	20	76.6	60	Supplemental
"	"	0.058	30	63.3	65	Antagonism
"	"	0.068	40	53.3	70	Antagonism
"	"	0.079	50	60.0	75	Antagonism

Table 8. Mortality of *S. litura* caterpillars caused by sublethal doses of carbaryl and NPV (LC_{50}) combinations.

Treatments		Per cent mortality at the end of (days)										
NPV (PIBs/ml)	Carbaryl (%)	1	2	3	4	5	6	7	8	9	Total*	MTLD (Days)
8.62×10^6 (LC_{50})	0.09 (LC_{10})	10.0	10.0	16.6	16.6	26.6	40.0	40.0	43.3	53.3	53.3	5.5
"	0.11 (LC_{20})	13.3	16.6	26.6	26.6	33.3	40.0	43.3	43.3	56.6	56.6	5.0
"	0.13 (LC_{30})	33.3	36.6	36.6	40.0	46.6	56.6	63.3	63.3	70.0	70.0	3.7
"	0.15 (LC_{40})	23.3	33.3	33.3	53.3	66.6	73.3	73.3	76.6	76.6	76.6	3.4
"	0.17 (LC_{50})	33.3	40.0	40.0	50.0	60.0	66.6	70.0	73.3	80.0	80.0	3.6

* No mortality was noted in control

Fig.7 Mortality of _S. litura_ caterpillars caused by combinations of NPV and carbaryl.

Fig.8 Effect of combinations of NPV and carbaryl on _S. litura_ caterpillars.

A - NPV LC_{50} (8.62×10^{6} PIBs/ml) + Carbaryl LC_{10} (0.09%)

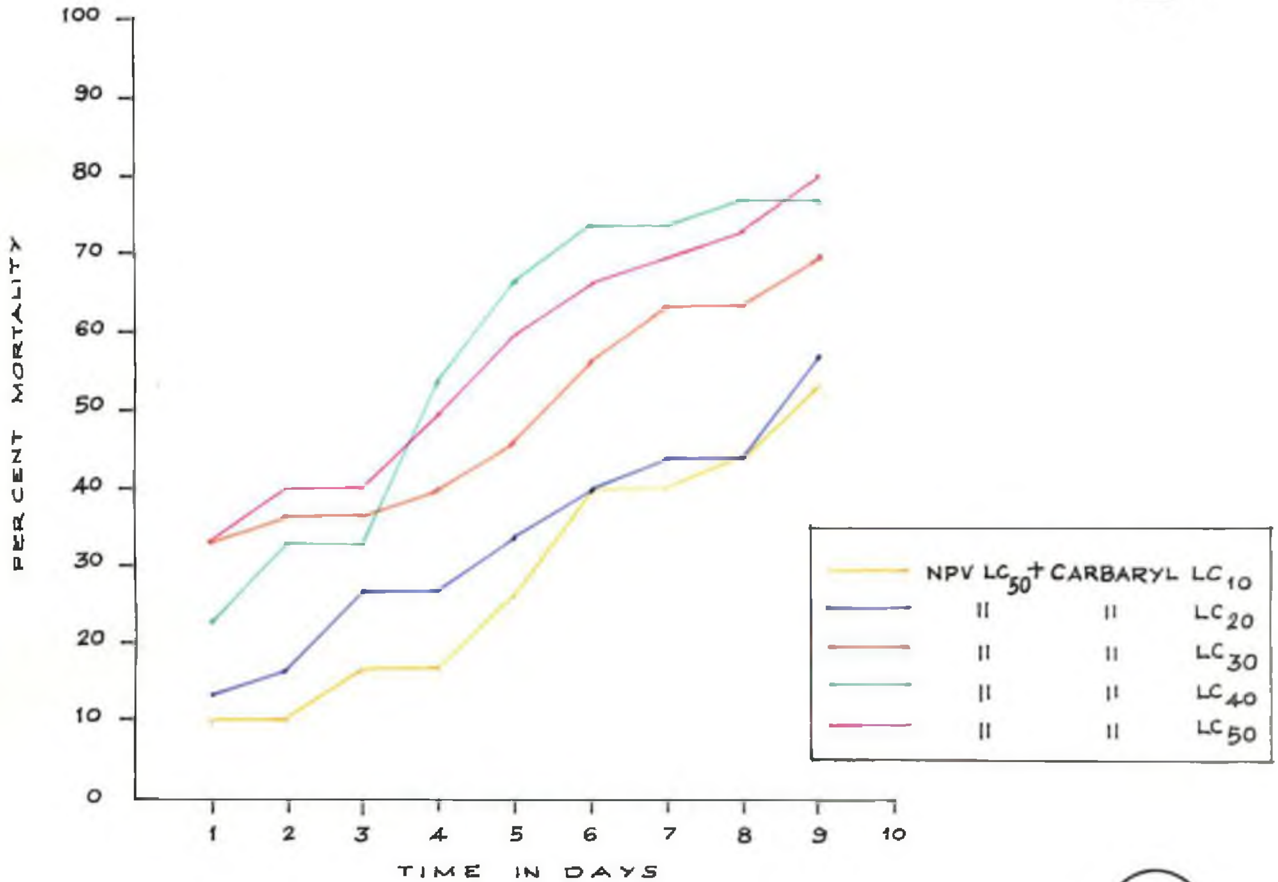
B - " " LC_{20} (0.11%)

C - " " LC_{30} (0.13%)

D - " " LC_{40} (0.15%)

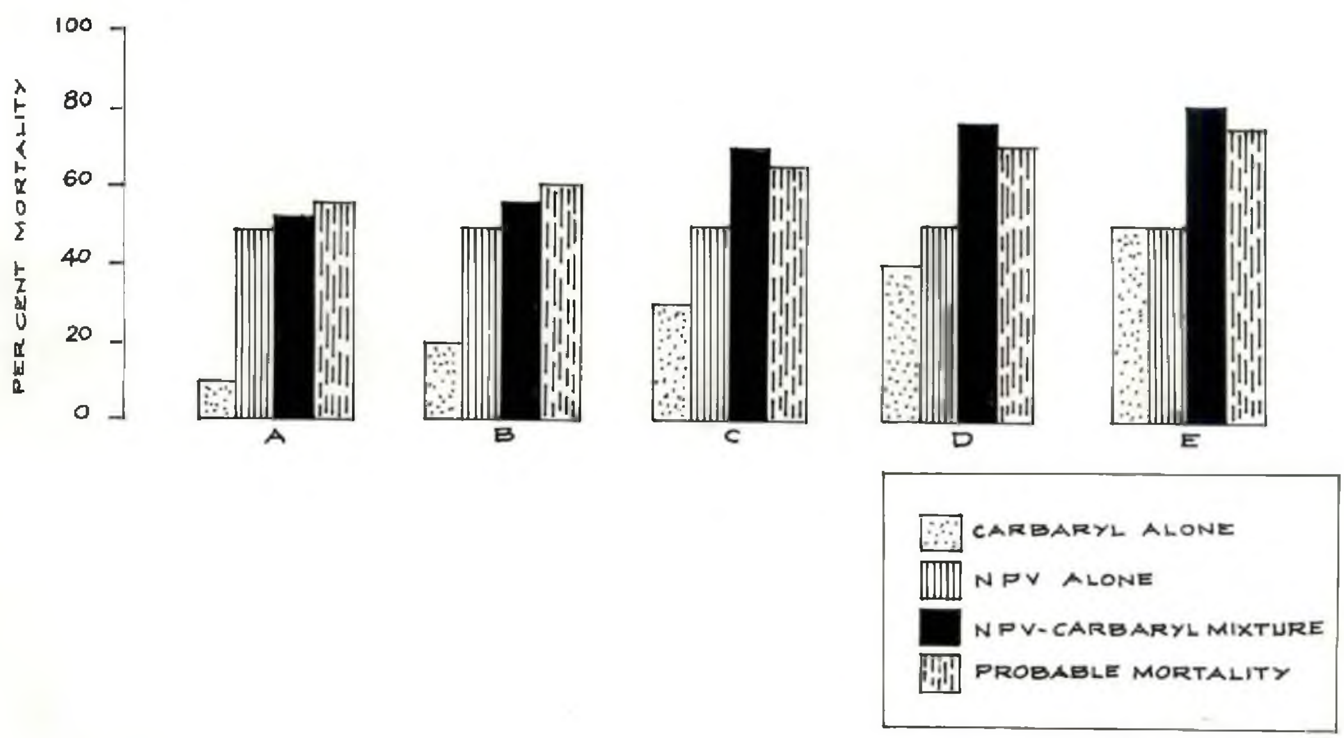
E - " " LC_{50} (0.17%)

FIG. 7



PER CENT MORTALITY
100
90
80
70
60
50
40
30
20
10
0
1
2
3
4
5
6
7
8
9
10
TIME IN DAYS
NPV LC50 + CARBARYL LC10
" " LC20
" " LC30
" " LC40
" " LC50

FIG. 8



PER CENT MORTALITY
100
80
60
40
20
0
A
B
C
D
E
CARBARYL ALONE
NPV ALONE
NPV-CARBARYL MIXTURE
PROBABLE MORTALITY

All the virus-insecticide mixtures showed mortalities ranging from 10 to 33.3 per cent on the first day following the treatment. In general there was a gross correspondence between the graded doses of virus-insecticide mixtures and per cent mortalities caused by them. The total mortality obtained varied from 53.3 per cent for the lowest dose combination and 80.0 per cent for the highest dose combination. Significant difference in per cent mortality was observed only between treatments using the highest and lowest doses of the insecticide. The lowest MTLD values 3.7, 3.4 and 3.6 days were obtained when LC_{30}, LC_{40} and LC_{50} of the insecticide were used.

The effects of combining NPV and carbaryl at sublethal concentrations in comparison with the effect of the individual components is presented in Table 9 and Fig. 8. Subadditive effect was noticed in three combinations where a higher dose of insecticide was mixed with LC_{50} concentration of NPV while an antagonistic effect was observed when LC_{10} and LC_{20} of carbaryl was mixed with NPV at LC_{50} concentration.

<u>Joint action of NPV and monocrotophos on caterpillars of *S. litura*.</u>

The effect of combinations of NPV and sublethal concentrations of monocrotophos on caterpillars of

Table 9. Effect of the combinations of NPV and carbaryl on *S. litura* caterpillars.

NPV		Carbaryl		NPV-Carbaryl mixture mortality (%)	Probable mortality by combined action (%)	Effect
Concentration (PIBs/ml)	Mortality (%)	Concentration (%)	Mortality (%)			
8.62×10^6	50	0.09	10	53.3	55	Antagonism
"	"	0.11	20	56.6	60	Antagonism
"	"	0.13	30	70.0	65	Subadditive
"	"	0.15	40	76.6	70	Subadditive
"	"	0.17	50	80.0	75	Subadditive

S. litura in comparison with the individual effects of NPV at LC_{50} and monocrotophos at sublethal concentrations is presented in Table 10 and Fig. 9.

It could be seen that mortality of the caterpillars started from first day onwards. There was a general increase in mortality with increase in graded dose of sublethal concentrations of the insecticide except for the combinations in which LC_{40} and LC_{50} doses of the insecticide were used. The combinations involving LC_{30} and LC_{40} of the insecticide and LC_{50} of NPV recorded the highest mortality of 66 per cent, while combination between LC_{10} of the insecticide and LC_{50} of the virus recorded the lowest mortality of 55 per cent. However, all the treatments were on par with each other. A correspondence was noticed between MTLD and increase in dose of the insecticide in the combination, the lowest being 2.1 days obtained when highest dose of insecticide was used.

The effects of combining NPV with monocrotophos together with the categorisation of the different effects are presented in Table 11 and illustrated in Fig. 10. It was observed that when the lowest dose of insecticide was combined with NPV an independent action was obtained.

Table 10. Mortality of *S. litura* caterpillars caused by sublethal doses of monocrotophos and NPV (LC_{50}) combinations.

Treatments		Per cent mortality at the end of (days)										
NPV (PIBs/ml)	Monocrotophos (%)	1	2	3	4	5	6	7	8	9	Total*	MTLD (Days)
8.62×10^6 (LC_{50})	0.019 (LC_{10})	6.66	6.66	6.66	6.66	13.3	23.3	23.3	26.6	55.0	55.0	7.0
"	0.026 (LC_{20})	10.0	13.3	13.3	13.3	23.3	36.6	36.6	46.6	59.0	59.0	5.8
"	0.033 (LC_{30})	10.0	10.0	10.0	10.0	16.7	33.3	33.3	40.0	66.0	66.0	6.6
"	0.04 (LC_{40})	23.3	56.6	56.6	56.6	56.6	59.9	59.9	59.9	66.0	66.0	2.6
"	0.049 (LC_{50})	26.6	46.6	46.6	56.6	56.6	59.0	59.0	59.0	59.0	59.0	2.1

* No mortality was observed in control.

Table 11. Effect of the combinations of NPV and monocrotophos on S. *litura* caterpillars.

NPV		Monocrotophos		NPV-Monocrotophos mixture mortality (%)	Probable mortality by combined action (%)	Effect
Concentration (PIBs/ml)	Mortality (%)	Concentration (%)	Mortality (%)			
8.62×10^6	50	0.019	10	55	55	Independent
"	"	0.026	20	59	60	Antagonism
"	"	0.033	30	66	65	Subadditive
"	"	0.04	40	66	70	Antagonism
"	"	0.049	50	59	75	Antagonism

Fig. 9 Mortality of *S. litura* caterpillars caused by combinations of NPV and Monocrotophos.

Fig. 10 Effect of combinations of NPV and monocrotophos on *S. litura* caterpillars.

A - NPV LC_{50} (8.62×10^6 PIBs/ml) + monocrotophos LC_{10} (0.019%)

B - " " LC_{20} (0.026%)

C - " " LC_{30} (0.033%)

D - " " LC_{40} (0.04%)

E - " " LC_{50} (0.049%)

FIG. 9

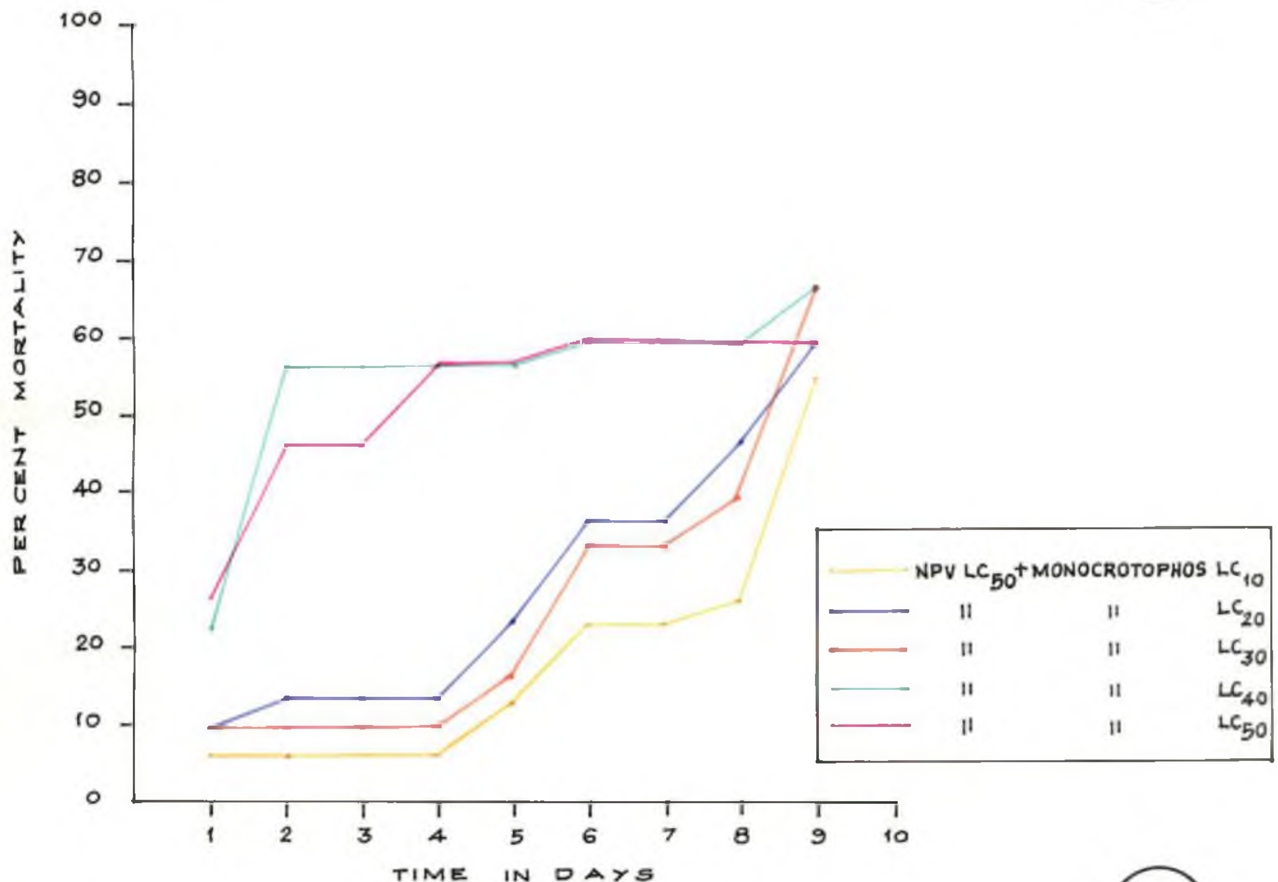
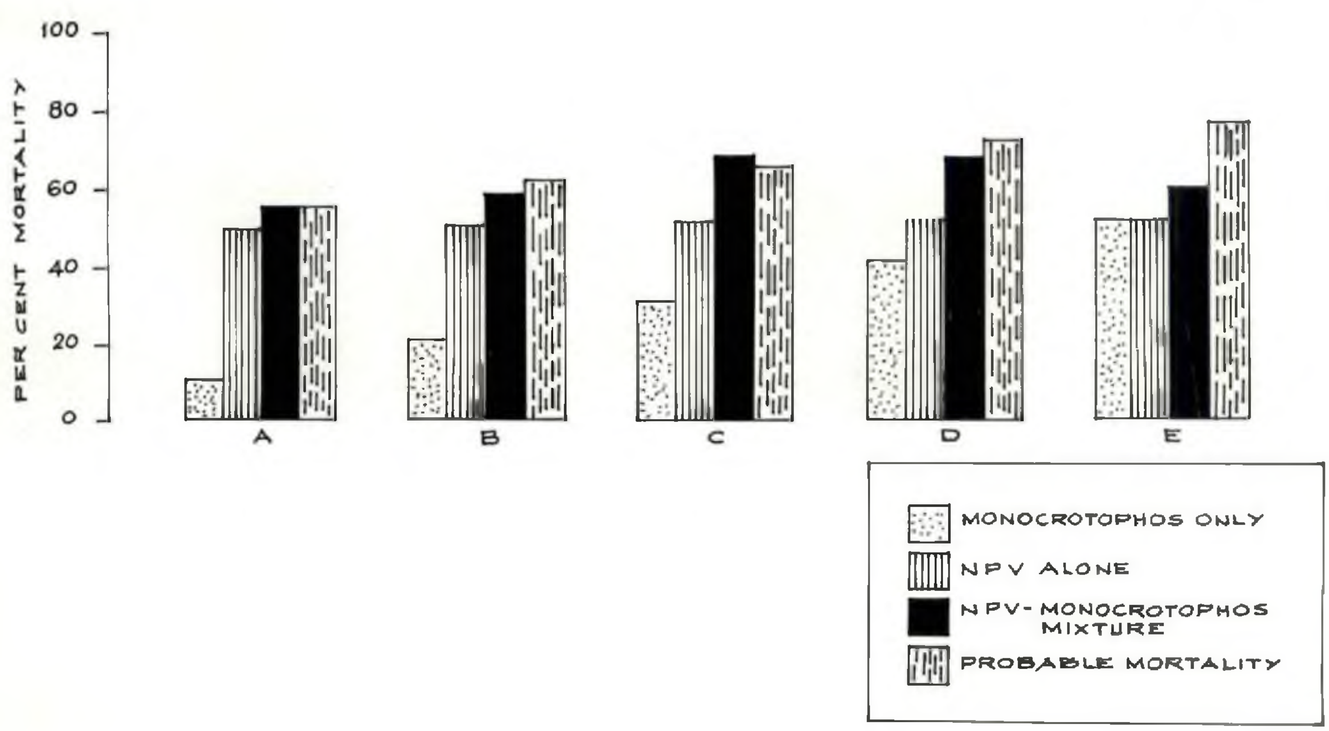

PER CENT MORTALITY
TIME IN DAYS
NPV LC_{50} + MONOCROTOPHOS LC_{10}
" " LC_{20}
" " LC_{30}
" " LC_{40}
" " LC_{50}

FIG. 10


PER CENT MORTALITY
A
B
C
D
E
MONOCROTOPHOS ONLY
NPV ALONE
NPV- MONOCROTOPHOS MIXTURE
PROBABLE MORTALITY

When LC_{20}, LC_{40} and LC_{50} doses of insecticide were mixed with LC_{50} of NPV the effect was antagonistic showing incompatibility between virus and the insecticide at these levels.

Effect of application of NPV-insecticide mixtures for the control of *Spodoptera litura*.

The combinations found promising under laboratory studies were tested under field conditions. The results of the trials conducted in the field are presented below.

a) Simultaneous application.

The results of simultaneous application of NPV and insecticides on the larvae of *S. litura* are furnished in Table 12. NPV with quinalphos at LC_{10} and LC_{50} levels recorded 71.1 per cent and 77.8 per cent mortality. When LC_{30} and LC_{40} levels of permethrin were combined with LC_{50} of NPV the mortality recorded were 84.40 and 82.2 per cent respectively. It can be seen that both insecticides at the levels tested maintained their synergistic effect in the field also.

b) Sequential application.

Data presented in Table 13 show the effect of different methods of application of NPV and insecticides on the larvae of *S. litura*.

Table 12. Mortality of S. litura caterpillars caused by application of NPV-insecticide combinations on cowpea plants.

Insecticide	Doses NPV	Doses Insecticide	Per cent mortality*	Probabale mortality by combined action	Type of synergism
	-	LC_{10}	8.87		
	-	LC_{50}	40.0		
a) Quinalphos	LC_{50}	-	31.11		
	LC_{50} +	LC_{10}	71.1	37.22	Potentiating
	" +	LC_{50}	77.8	58.66	"
	-	LC_{30}	17.8		
	-	LC_{40}	26.7		
b) Permethrin	LC_{50}	-	37.8		
	LC_{50} +	LC_{30}	84.4	48.87	Potentiating
	" +	LC_{40}	82.2	54.40	"

* Mean value of three replications corrected for control mortality.

Table 13. Mortality of *S. litura* caterpillars caused by different methods of applications of NPV and insecticides on cowpea plants.

Combinations	Per cent mortality of larvae*		
	Simultaneous application of NPV and Insecticide	Sequential application of NPV followed by insecticide.	Sequential application of insecticide followed by NPV
1) LC_{10} Quinalphos and LC_{50} NPV	71.1	77.8	73.3
2) LC_{50} Quinalphos and "	77.8	84.4	68.9
3) LC_{30} Permethrin and "	84.4	84.4	88.9
4) LC_{40} Permethrin and "	82.2	88.8	91.1

* Mean values of three replications corrected for control mortality.

The sequential application of LC_{50} virus followed by LC_{10} quinalphos caused a total mortality of 77.8 per cent while the application of the same dose of virus followed by LC_{50} quinalphos recorded 84.4 per cent mortality. When quinalphos at LC_{10} and LC_{50} were applied first followed by NPV the mortalities recorded were 73.3 and 68.9 per cent only.

The sequential application of NPV followed by permethrin at LC_{30} level recorded a mortality of 84.4 per cent while in the treatment involving LC_{40} of permethrin it was 88.8 per cent. The highest mortalities of 88.9 and 91.1 per cent were recorded when permethrin at LC_{30} and LC_{40} were applied first followed by virus application.

Statistical analysis of the data showed no significant difference between treatments.

DISCUSSION

DISCUSSION

Compatibility of viruses with chemical pesticides especially insecticides is of special concern because of increasing interest in using them together in integrated pest management. Chemical pesticides and microbial agents used in combination are reported to be effective in field due to the synergistic effect. Such combinations may also help to reduce the incubation period of insect viruses. The integration of chemical insecticides with biological ones is recognized as an effective means to reduce the quantity of insecticide used in field which would in turn reduce environmental pollution and harmful effects on non-target fauna. Biological and chemical insecticides can be used in simultaneous or sequential combinations. The nature of chemical insecticides, dosage levels of biological and chemical insecticides and interval of sequential application may affect the type of synergism. Though considerable information has been gathered on the use of pathogenic fungi as well as bacteria combined with insecticide similar studies on insect viruses are limited. Hence investigations were taken up to gather information on the possibility of combining NPV of *S. litura* with insecticides.

First attempt was to establish the dose-mortality relationship between the NPV and third instar larvae of S. litura using the bioassay technique. The LC_{50} of the virus for the third instar larvae of S. litura was found to be 8.62×10^6 PIBs/ml. Abul-Nasr (1956) reported that suspensions of 5-10 million polyhedra were sufficient to infect the third instar larvae of S. litura. Pawar and Ramakrishnan (1975) reported an LC_{50} of 7.97×10^6 PIBs/ml for 5 day old larva of S. litura. The LC_{50} obtained in the present study was in agreement with the earlier studies.

Bioassay of quinalphos, permethrin, BHC, carbaryl and monocrotophos was done on the larvae of S. litura. Dosage mortality relations were worked out for each insecticide tested and regression equations were computed. These regression equations were used in calculating the sublethal doses ranging from LC_{10} to LC_{50} for the various combinations with the virus in the subsequent studies.

Quinalphos when combined with NPV of S. litura showed an increase in the percentage mortality of the larvae with increase in the dose of the insecticide. But the synergistic effect was found weakened with increase in concentration of the insecticide. The combination between LC_{10} of the insecticide and LC_{50} of the virus produced potentiation, but the effect decreased to supplemental level at LC_{20} and LC_{30} of the insecticide and to subadditive level in combinations involving LC_{40} and LC_{50} of the insecticide. The subadditive effects at higher concentrations of the insecticide can be attributed to a suppressive effect of the insecticide on viral activity. Susamma Mathai (1982) also obtained similar results by combining NPV of S. mauritia with quinalphos at sublethal doses. The initial mortality was high in treatments receiving higher levels of insecticides. But in the combination between LC_{10} of insecticide and LC_{50} of the virus almost 75 per cent of the total mortality was observed after the third day which could be due to the enhanced activity of the virus. Benz (1971) also reported similar synergistic effect on virus by sublethal doses of insecticides where the insecticides may cause stress on insects which in turn may become conducive to viral infections. The mean time to larval death (MTLD) was 5.8 days in this treatment as compared to 7 days when virus alone was used. This reduction in MTLD indicate the synergistic effect of the sublethal dose of insecticide on the virus.

Studies on the joint action of permethrin and the virus showed synergism in all combinations ranging from subadditive to potentiating effect. The lowest sublethal dose of the insecticide produced supplemental synergism while the LC_{50} dose of the insecticide produced only a subadditive effect. LC_{30} and LC_{40} of the insecticide produced true synergism leading to potentiation on viral activity. Susamma Mathai (1982) reported synergism ranging from supplemental to subadditive effect when the NPV of S. mauritia was combined with permethrin at sublethal doses. Komolpith and Ramakrishnan (1978) reported that permethrin at 5 ppm combined with NPV of S. litura produced potentiating effect. The MTLD was considerably reduced in all combinations which clearly indicate that the viral activity was hastened by the insecticide especially at the LC_{30} and LC_{40} levels. The very low MTLD when LC_{50} doses of the insecticide and virus were combined might be due to the relatively high toxicity of the insecticide at this level.

Combinations of BHC and virus were showing supplemental synergism at the LC_{10} and LC_{20} levels but at higher doses of BHC there was an antagonistic effect on viral activity. At lower doses the insecticide had acted as a stress factor making the insect more susceptible to viral invasion. The decrease in MTLD over that of virus alone can be attributed to synergistic action of the insecticide at these levels. Komolpith and Ramakrishnan (1978) found that γ-BHC in combination with S. litura NPV produced synergistic effect at slightly lethal doses of 50 and 20 ppm, but the synergistic effect was reduced at lower doses. The present results are not in agreement with this.

Effects produced by the combined activity of NPV and carbaryl could be categorised as antagonistic and subadditive synergism. The antagonistic effect observed at the two lower concentrations of carbaryl was a weak type. Similarly the subadditive effects observed in the combinations using higher doses of insecticide were also nominal. These results show that both the components were acting almost independently of each other. Ignoffo and Montoya (1966) also obtained only slight synergistic effect by combining carbaryl with NPV of Heliothis zea. It is evident from the results that no additional advantage in pest suppression could be obtained by combining carbaryl and the virus.

There was only slight difference between the actual mortality obtained in the combinations between lower doses of monocrotophos and virus which indicate that both components have acted almost independently at these levels.

Quinalphos when applied simultaneous with NPV in the field retained the potentiating effect at LC_{10} and LC_{50} levels. The results of the sequential application of virus and insecticide showed that post infection treatment of quinalphos recorded mortalities higher than that obtained when the virus and insecticide were applied together. At 48 hours after virus application the virus multiplication might have already started making the larvae more vulnerable to the knockdown action of insecticide. Such activation of insecticide by virus has been reported earlier in the case of NPV of cabbage looper in which prior subacute infections by NPV increased the susceptibility of larvae to endrin, endosulfan and trichlorphon (Benz, 1971). The total mortality obtained is higher than that obtained in the simultaneous application of insecticide and virus. The post infection treatment with LC_{50} of quinalphos recorded 84.4 per cent larval mortality showing that it is effective in controlling the pest in the field. Savanurmath and Mathad (1982) found that application of LC_{25} and LC_{50} virus followed by LC_{25} endosulfan yielded high virus disease incidence in the larvae of <u>Mythimna separata</u>. When virus was applied 48 hours after the treatment with quinalphos the per cent mortality in both cases was lower than that in the post infection treatments. The post-infection treatment of LC_{10} quinalphos recorded 77.8 per cent mortality while prior application of the same dose could give only 73.3 per cent mortality. It appears that the mild stress exerted by prior application of LC_{10} of the insecticide did not predispose the insect to viral infection and cause heavy mortality due to the disease in later stages. The pre-treatment with LC_{50} quinalphos recorded only 68.9 per cent larval mortality. While post treatment of the same dose recorded 84.4 per cent larval mortality. It is possible that the pre-treatment with LC_{50} quinalphos caused high initial mortality giving less opportunity to the virus to invade the host and cause death. It also might have caused changes in the gut epithelium and other tissues and thereby rendering them unsuitable for viral invasion and multiplication (Lal et al. 1970).

Permethrin produced potentiating synergism at LC_{30} and LC_{40} when combined with NPV and applied simultaneously. The results of simultaneous and sequential application of permethrin with virus show that the sequential application is better than applying the virus and insecticide together. Sequential application involving pretreatment of permethrin gave better results than post infection treatment of the insecticide. Application of LC_{40} permethrin followed by LC_{50} of the virus was the most promising giving 91.1 per cent larval mortality. It shows that the mild stress produced by the insecticide made the insect more susceptible to viral infection as suggested by Savanurmath and Mathad (1981).

In the present studies synergistic effects leading to potentiation could be observed with quinalphos and permethrin and it was found to be dose dependent. The dose dependent nature of potentiation when biological and chemical insecticides were mixed was reported earlier by Benz (1971). Reports of synergism are those of NPV of *Lymantria dispar* by sublethal doses of DDT and lindane (Vasiljevic, 1967); NPV of *Heliothis zea* by carbaryl (Ignoffo and Montoya 1966); NPV of *Idaea seriata* by carbaryl, derris, DDD, DDT, nirosan and imidazole, NPV of *T. ni* by endrin, endosulfan and trichlorphon (Benz, 1971); NPV of *S. litura* by DDT, lindane, malathion and pyrethin (Komolpith and Ramakrishnan, 1978) and NPV of *Mythimna separata* by fenitrothion and endosulfan (Savanurmath and Mathad 1981, 1982).

It was evident from the results that quinalphos and permethrin are the most promising insecticides for combined treatment with NPV of *S. litura*. Both the insecticides gave potentiating effects in the field also. Post-infection application of quinalphos was found to be more promising than pre-treatment of insecticide followed by the virus while in the case of permethrin pretreatment of the insecticide was found to be more effective. However, while taking into consideration the cost involved in sequential application of virus and insecticide simultaneous application of both components will be more economic in the field.

SUMMARY

SUMMARY

Studies were conducted to evaluate the effectiveness of combining various insecticides viz., quinalphos, permethrin, BHC, carbaryl and monocrotophos with the nuclear-polyhedrosis virus of Spodoptera litura (Fabricius).

The LC_{50} values of the virus and the insecticides to third instar larvae were determined using the bioassay technique. The LC_{50} of the virus was found to be 8.62×10^6 PIBs/ml. Permethrin with an LC_{50} of 0.002728 per cent was the most toxic to S. litura.

Laboratory studies on the combined application of sublethal doses of insecticides and LC_{50} of virus showed that quinalphos and permethrin are the most promising for combined application with the virus.

Quinalphos at LC_{10} produced potentiating effect when combined with the virus, while at higher doses it produced only supplemental and sub-additive effects. Permethrin at LC_{30} and LC_{40} when combined with virus had a potentiating effect, while at higher and lower doses the synergism was seen weakened.

When BHC and virus were combined an antagonistic effect was observed at higher doses of the insecticide while carbaryl in combination with the virus produced the antagonistic effect at lower doses. Monocrotophos also showed antagonistic effect when combined with virus.

Quinalphos and permethrin when combined with virus and applied simultaneously showed their potentiating effect in the field also. The results of sequential application of quinalphos and virus showed that post-infection treatment of the insecticide after 48 hours of virus application gave better control of the pest than pretreatment with the insecticide. But in the case of permethrin pretreatment with the insecticide followed by virus application after 48 hours was found to be more effective.

Considering the cost of sequential application, simultaneous application of virus and insecticide will be more economic.

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JOINT ACTION OF NUCLEAR POLYHEDROSIS VIRUS OF *Spodoptera litura* (Fabricius) WITH INSECTICIDES AND ITS APPLICABILITY IN PEST CONTROL

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ABSTRACT

Experiments were conducted to study the joint action of nuclearpolyhedrosis virus (NPV) of Spodoptera litura (Fabricius) and five insecticides viz., quinalphos, carbaryl, BHC, monocrotophos and permethrin. The investigations carried out included bioassay of the virus and insecticides on third instar larvae of S.litura, effect of different combinations of the virus and insecticides on larval mortality and the effect of simultaneous and sequential application of virus and insecticides for the control of pest.

The LC_{50} of the virus and that of the insecticides were determined by bioassay. Uniform sized leaf-discs of castor (Ricinus communis) sprayed with suspensions of virus and insecticides under a Potter's tower were fed to the third instar larvae. The larval mortalities were recorded. Dose-mortality relations were worked out by probit analysis. Joint action was assessed by combining sublethal doses of various insecticides with LC_{50} dose of virus. Bioassay technique was utilized for this also. The joint action of virus and insecticides was classified based on the criteria proposed by Benz (1971). Combinations found promising were used in field studies involving simultaneous and sequential application of virus and insecticide.

LC_{50} of virus was found to be 8.62×10^6 PIBs/ml. Among the insecticides permethrin with an LC_{50} of 0.002728 per cent was the most toxic to the larvae.

Quinalphos and permethrin were found to be most promising for combined application with the virus. Quinalphos at LC_{10} level produced potentiating effect when combined with the virus while at higher doses it produced only supplemental and sub-additive effects. Permethrin at LC_{30} and LC_{40} levels when combined with virus showed potentiating effect, but at higher and lower doses the synergism was weakened. BHC showed supplemental synergism when LC_{10} and LC_{20} doses were combined with virus while it showed antagonism at higher doses. Carbaryl produced antagonistic effect at lower doses but the effect was subadditive at higher doses. The joint action between virus and monocrotophos ranged between weak subadditive synergism and antagonism.

Quinalphos and permethrin in combination with virus and applied simultaneously retained their potentiating effect in the field also. The results of sequential application of quinalphos and virus showed that post infection treatment of insecticide after 48 hours of virus application gave better control of the pest than pre-treatment with the insecticide. In the case of permethrin pretreatment with the insecticide followed by virus application after 48 hours was found to be more effective. However, while taking into consideration the cost of sequential application of virus and insecticide simultaneous application of these two components will be more economic.