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Study the Toxicity of Three Benzothiazole Compounds Formulated as 10 % Suspension Concentrate against Cotton Leafworm *Spodoptera littoralis* (Boisd)

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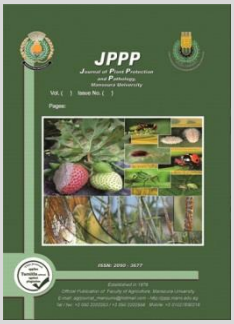


ABSTRACT

Three compounds of benzothiazole were synthetized as active ingredient and prepared as 10 % suspensions concentrate (SC). They were evaluated against the second and fourth instar larvae of the cotton leafworm *S. littoralis (Boisd.)*, under laboratory conditions. LC_{50} values showed that formulation (1) gave the highest toxic effect against 2nd and 4th instar larvae after 48 hrs from treatment and gave the best effect as antifeedant followed by formulation (3) and formulation (2). Semi field experiment was done to evaluate the initial and latent effects of the formulated compounds compared with commercial insecticide chlorpyrifos 48% EC (Dursban), the results showed that formulation (1) gave 100 % prevention of adult emergency due to the high initial and latent effects and lowest pupation percentage as the same as chlorpyrifos followed by formulation (3) and formulation (2) with the 2nd and the 4th instar larvae. Whereas Formulation (1) at 1 % is already as the same of recommended insecticide chlorpyrifos, therefore it could be recommended to use the new formulations as alternative of hazard conventional insecticides in controlling cotton leafworm in different crops after conducting the other necessary open field experiments.

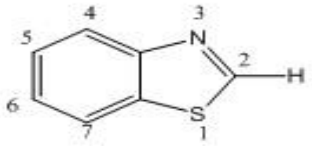
Keywords: Benzothiazole; Suspension Concentrate Formulation, Cotton leafworm

INTRODUCTION

Heterocyclic compounds containing nitrogen and sulphur atoms have been identified to have the most significant biological activities Wang *et al*., (2011). It has an important pharmacophore effects as benzothiazole and its novel analogs which have been found to have a wide variety of therapeutic activities in medicinal chemistry Kumbhare *et al*., (2012) such as in anticancer Jin *et al*., (2006), antitumor Chen *et al*., (2008), antimicrobial Sahu *et al*., (2012)

Benzothiazole is a six-membered bicyclic heteroaromatic compound in which benzene ring is fused to the 4- and 5-positions of thiazole ring. Benzothiazoles are found in marine as well as terrestrial natural compounds in a very less amount but have considerable pharmacological effects, where they act as aroma constituents of tea leaves and cranberries which are produced by fungi named *Aspergillus clavatus* and *Polyporus frondosus.*

Various benzothiazole derivatives such as 2-aryl benzothiazole are in the eyes of most scientists due to its diverse structure and its uses as radioactive amyloid imaging agents.



1, 3- Benzothiazole

It is reported that derivatives of benzothiazole have antimicrobial activity against various types of gram positive and gram negative bacterias (e.g., *E. coli, Pseudomonas aeruginosa, Enterobacter Staphylococcus epidermis, etc.*). Bondock *et al*., (2010).

The cotton leaf worm, *Spodoptera littoralis* Boisd (Lepidoptera: Noctuidae) is the most common, serious and devastative pests which attack large scale of economic crops as cotton, clover, maize and different vegetable crops. To overcome the risk of this pest, the farmers heavily used synthetic chemical insecticides which lead to appearance of impedance pests' strains and its residue caused defilement to circumference which effect all living organisms (Abo El-Ghar *et al*., 1986).

The developed world has progressed to develop eco-friendly formulations to meet the needs for operator as well as environmental safety or to improve the bio-efficacy and persistence of pesticides. These formulations would not only replace toxic, non-degradable inert ingredients/adjuvants of the conventional formulations but also increase the activity of the products through incorporating latest technologies like size reduction (Wettable Powder to Suspension Concentrate, Soluble Liquid to microemulsion), increased coverage of applied surface area (Emulsifiable Concentrates to ME formulations), reduced wastage (Dust/WP to controlled release formulations) and dose rates to improve food and environment quality with minimum pesticide residues. (Hazra 2015)

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The aim of the present work was using the local synthesis of benzothiazole derivatives and formulating it in a suitable formulation form and evaluation their pesticidal efficacy against the 2nd and 4th instar larvae of cotton leafworm.

MATERIALS AND METHODS

1 Fine Chemicals:

a) Synthetized compounds:

Compound (1): 2-(benzo[d]thiazol-2-yl) acetonitrile

Compound (2): 4-amino-5-(benzothiazole-2-yl)-3-phenylthiazole-2- thione

Compound (3): benzo[d]thiazole-2-thiol.

b) Traditional insecticide: chlorpyrifos 48 % EC (Dursban): Central Agricultural Pesticides Lab. (CAPL), Agriculture Research Center (ARC), Dokki, Giza, Egypt

c) Solvents: acetone, xylene, dimethyl formammide, chloroform, dichloromethane, sulphur, phenylisothiocynate, aniline, carbon disulfide and triethyl amine were supplied by El- Gomhoria Co., Cairo, Egypt.

d) Surface active agents: sodium dodecyl sulfate, span 20, toximol 500 were supplied by El- Gomhoria Co., Cairo, Egypt.

e) Poly ethylene glycol 600 mono- lurate were supplied by the Egyptian Starch, Yeast and Detergents Co., Alexandria, Egypt.

f) Sticking agent: Arabic gum was supplied by El-Gomhoria Co., Cairo, Egypt.

2. The physico- chemical properties of the basic formulation components:

1. Active ingredient:

a) Solubility**:** It was determined by measuring the volume of distilled water, acetone and xylene for complete solubility or miscibility of one gram of active ingredient at 20 ˚C (Nelson and Fiero, 1954). The % solubility was calculated according to the following equation:

$$\% \text{ solubility} = W/V \times 100$$

Where; W = active ingredient weight, V = volume of solvent required for complete solubility.

b) Free acidity or alkalinity: It was determined according to the method described by WHO specification (1979).

2. Surface active agents:

a) Surface tension: It was determined by using surface tensiometer for solutions containing 0.5 % (W/V) surfactant according to ASTM D-1331 (2001).

b) Critical micelle concentration (CMC): The concentration in which the surface tension of solution doesn't decrease with further increase in surfactant concentration, (CMC) of the tested surfactants was determined according to the method described by (Osipow, 1964).

c) Hydrophilic-lipophilic balance (HLB): The solubility of surfactant in water is considered as approximate guide to its hydrophilic-lipophilic balance, was determined according to the method described by (Lissant *et al*., 1971).

d) Free acidity or alkalinity: It was determined by the same method described before.

3. Preparation of the synthetized benzothiazole compounds as 10% suspension concentrates (SC).

Suspension concentrate (SC) formulations are solid active ingredient dispersed in water. It provides good safety, user convenience and effectiveness when compared to other formulation types. This type of formulation has been developed for active ingredients that are not soluble in oils or water.

The new suspension concentrate formulation was obtained through trials as follow:

Different weights of active ingredients after grinding and sieving were added to other different weights from wetting and dispersing agents with different percentages of water and sticking agent. Then the mixture was stirred using magnetic stirrer to ensure homogeneity. Suspensbility test was carried out for all prepared formulations according to CIPAC MT 46.1 (2002) to judge on the success of formulations.

4. Determination of the physico- chemical properties of the prepared suspension concentrates formulation (SC).

a) Suspensibility: It was determined according to CIPAC MT 46.1 (2002)

b) Foam: it was measured according to CIPAC (2002).

c) Free acidity or alkalinity: It was measured as mentioned before.

e) Stability at elevated temperature 54 ± 2 ºC (accelerated storage): It was measured according to **CIPAC (2002)**

5. Determination of the physico-chemical properties of the spray solution of the local prepared formulation at the field dilution rate.

a) Surface tension: It was determined as mentioned before.

b) PH: It was determined by using Cole-Parmer PH conductivity meter 1484-44 according to Dobrat and Martijn (1995).

c) Viscosity: It was determined by using Brookfield viscometer Model DVII+Pro, where centipoise is the unit of measurement according to ASTM D-2196 (2005)**.**

d) Electrical Conductivity: It was determined by using Cole-Parmer pH/Conductivity meter 1484-44, where µmhos is the unit of electrical conductivity measurements according to Dobrat and Martijn (1995).

6. Reared culture:

A laboratory reared culture of Egyptian cotton leafworm according to El- Defrawi, (1964) was used to determine the insecticidal activity of the tested compounds on the 2nd and 4th instar larvae of *S. littoralis*

Laboratory experiment (Toxicity test):

1. Toxic effect of the tested Formulations against the 2nd and the 4th instar larvae:

The toxic effect of the tested formulated compounds was assessed against 2nd and 4th instar larvae. Serial successive concentrations of formulations were used 250, 500 and 1000 ppm. Castor bean leaves were dipped for 15 seconds in each concentration then picked up and left to dry. The treated leaves were offered to the 2nd and the 4th instar larvae for 48 hrs then replaced by untreated leaves for 24 hrs. Mortality percentages were recorded after 72 hrs. and corrected according to natural mortality (Abbot, 1925). To estimate the LC_{50} values, the corrected

mortality percentages were subject to probit analysis according to Finney (1952).

2. Antifeedant effect of formulations against the 2nd and the 4th instar larvae:

Antifeedant effect of formulations against the 2nd and the 4th instar larvae were determined by introducing weight of treated Caster been leaves with the concentrations mentioned before compared with untreated one. After 48 hrs of feeding, the remaining leaves were weighed in each replicate, then the consumed amount of leaves were calculated Waldbauer (1968)

% Antifeedent = Cc – Ct/Cc x 100

Where: Cc = Consumed amount in untreated and Ct = Consumed amount in treatment.

3.Semi field experiment:

The experiment was conducted according to the recommendation of agricultural pests, Ministry of agriculture and land Reclamation (1993) and Mohamed *et al*., (2001) but at small scale. Cotton plants were grown in three pots for each concentration and treatments and three pots as control.

Evaluation Procedures:

To investigate the initial and latent effects of the tested formulations against cotton leafworm, spraying was done on cotton plants after 50 days of planting using hand plastic 1L capacity sprayer at concentrations of 0.25, 0.5 and 1 % at June 2019. After spraying when plant became dry 6 leaves of each treatment were taken then transferred to the laboratory and introduced to the 2nd and the 4th instar larvae of cotton leafworm at constant conditions of 25 ± 2ºC and 70 ±5 RH, three replicates for each treatment, each one contain 20 larvae; for studying latent effect, other samples were taken from treated pots and introduced to larvae until pupal stage.

Mortality count was recorded each 2 days, and then mortality percentage was calculated. Developmental effect against both pupae and adult emergency was studied by recording total number of pupal percentages and percentage of adult emergency was calculating by the method described by El- Sisi and Farrag (1989) as follow:

% Pupation = No. of formed pupae / Initial No. of 2nd or 4th instar larvae x 100

% Adult emergency = No. of formed moth / Initial No. of 2nd or 4th instar larvae x 100

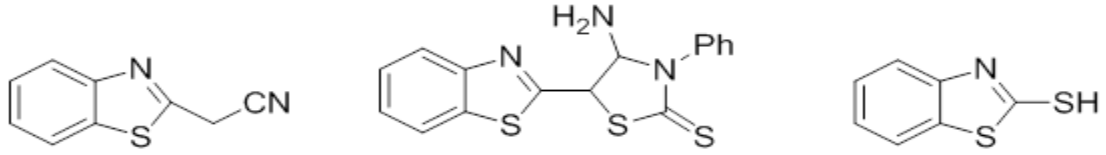
RESULTS AND DISCUSSION

1. Chemistry:

Compound (1) resulted from the reaction of 2-aminothiophenol with ethyl 2-cyanoacetimidate in chloroform or in dichloromethane afforded the formation of 2-(benzo[d]thiazol-2-yl) acetonitrile Ammar *et al*., (2002) and Toutchkine *et al*., (2007)

Compound (2) resulted from the reaction of compound (1) with sulphur metal and phenylisothiocyanate in DMF in the presence of TEA Zaki *et al*., (2006)

Compound (3) resulted from the reaction of aniline with carbon disulfide and sulfur Hamood (2006).



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2. Formulation:

The results in Table (1) showed that all compounds were insoluble in water and xylene but slightly soluble in dimethyl formammide and acetone. The three compounds showed low acidity values

Table 1. Physico- chemical properties of the synthesized active ingredients

Compounds	Solubility % (W/V)				Free acidity as % H_2SO_4	Melting point ºC
	water	xylene	Acetone	DMF		
1	No	No	3.3	16.7	0.098	100
2	No	No	No	5	0.392	250
3	No	No	10	16.7	0.098	247

As shown in Table (2), the physico- chemical properties of surface active agent namely sodium dodecyl sulfate, toximol 500, polyethylene glycol 600 mono-lurate, and span 20 were studied to determine if they were compatible with the physico- chemical properties of the locally synthetized active ingredients or not. According to HLB values, sodium dodecyl sulfate and polyethylene glycol 600 mono- lurate were considered as dispersing agent, their HLB values were more than 13 whereas the HLB values of span 20 and toximol 500 was (8 – 10), so they were considered as wetting agents. On the other hand all tested surface active agents' decreased surface tension from 72 for water to 37.3, 31, 28.5 and 27.6 dyne/cm in case of span 20, SDS, PEG 600 ML and toximol 500 respectively.

Table 2. Physico- chemical properties of surface active agents

Surface active agent	Surface tension (dyne/cm)	HLB	CMC %	Free acidity as % H_2SO_4	Free Alkalinity as % NaOH
SDS	31	17	0.3		0.026
Toximol 500	27.6	8-10	0.3	0.39	-
PEG 600 ML	28.5	14	0.4	0.61	-
Span 20	37.3	8 -10	0.3	0.15	-

SDS: sodium dodecyl sulfate
PEG 600 ML: poly ethylene glycol 600 mono-lurate.

Toximol 500, PEG 600 ML and span 20 recorded % free acidity as H_2SO_4 0.39, 0.61 and 0.15 respectively, whereas sodium dodecyl sulfate recorded slightly % free alkalinity 0.026 as NaOH. However SDS, toximol 500 and span 20 recorded the same CMC value 0.3 while PEG600 ML had 0.4 % as CMC value. From the above results, it could be concluded that, the tested surface active agents were suitable to prepare the synthetized benzothiazole derivatives as suspension concentrate (SC) because they

act as wetting agent in case of span 20 and toximol 500, and as dispersing agent in case of sodium dodecyl sulfate and PEG 600 ML .

Table (3) showed the Physico-chemical properties of the locally prepared 10 % suspension concentrate (SC) before and after accelerated storage at 54 ± 2 ºC for 3 days. It passed successfully all physical properties reported in addition it showed a slight decrease in suspensibility percentage and foam values after accelerated storage beside a slight increase in acidity values

Table 3. Physico-chemical properties of the locally prepared suspension concentrate (10 % SC) before and after accelerated storage.

Compounds	Before storage					After storage				
	% Suspensibility		Foam (cm^3)		Free acidity as % H_2SO_4	% Suspensibility		Foam (cm^3)		Free acidity as % H_2SO_4
	H.W	S.W	H.W	S.W		H.W	S.W	H.W	S.W	
1	91.4	92.8	2	0	0.68	90.7	91.4	1	0	0.71
2	92.5	93.4	3	1	0.49	91.7	92.5	2	1	0.68
3	94.6	96.4	2	0	0.54	93.2	95.3	1	0	0.54

H.W: Hard water (342 ppm as $CaCO_3$) S.W: Soft water (57 ppm)

Data in Table (4) showed the physico- chemical properties of spray solutions at the expected field dilution rate (0.5 %).

Table 4. Physico-chemical properties of spray solutions at field dilution rate (0.5 %).

Compounds	Viscosity (Centipoise)	Electrical conductivity (µ mhos)	pH	% Salinity	Surface tension (Dyne/cm)
1	1.96	438	6.72	0.2	38.4
2	1.98	452	6.77	0.2	40.5
3	2.5	447	6.63	0.2	38.9
water	1.0	417	7.15	0.2	72.0

The results showed that all formulated compounds have the same salinity value 0.2 % it considered low value and important for increasing biological efficacy. Also they gave acidic pH value. Formulation (2) gave the highest surface tension followed by Formulation (3) and Formulation (1) their values were 40.5, 38.9 and 38.4 dyne/cm respectively. Formulation (2) showed the highest electrical conductivity 452 µ mhos followed by Formulation (3) and Formulation (1) 447 and 438 µ mhos respectively. With respect to the viscosity, formulation (3) gave the highest value 2.5 centipoise followed by Formulation (2) 1.98 centipoise and Formulation (1) 1.96 centipoise. It was noticed that all compounds had viscosity value greater than water, which enhancement the biological efficacy by decreasing spray solution droplets size and increasing the treated surfaces wettability.

3. Bioassay:

a) Toxic effect:

Table (5) showed the toxicity of the prepared three formulations against the 2nd and the 4th instar Larvae of *S. littoralis* after 48 hrs under laboratory conditions. It was noticed that formulation (1) was considered the most toxic compound with the lowest LC_{50} value followed by formulation (3) and formulation (2) with LC_{50} values in case of 2nd instar Larvae were 423.12, 1221.3 and 1657.4 ppm respectively, but in case of 4th instar Larvae were 547.48, 1377.98 and 2522.3 ppm respectively. This means that the 2nd instar larvae was more sensitive than the 4th instar larvae for all tested formulation. These results agreed with (Hamouda, 2016) who reported that acrylonitrile derivative that was synthesized from benzothiazole-1,3-acetonitrile, and tested against cowpea aphid (*Aphis craccivora*) under laboratory conditions by slide dipping technique showed good aphicidal activity and its LC_{50} value was 614.33 ppm.

b) Antifeedant effect:

Data in Table (6) showed the antifeedant effect of the formulated compounds 10 % (SC) against 2nd and 4th instar larvae of *S. littoralis* under laboratory conditions..

Table 5. Toxicity of formulated benzothiazole Compounds on 2nd and 4th instar larvae of *S. littoralis*.

Compounds	2nd			4th		
	LC_{50}(ppm)	LC_{90}(ppm)	Slope ± SE	LC_{50}(ppm)	LC_{90}(ppm)	Slope ± SE
1	423.12	1419.50	2.438±0.327	547.48	1968.70	2.305±0.322
2	1657.40	10564.97	1.593±0.352	2522.30	18665.90	1.474±0.378
3	1221.30	8193.14	1.550±0.329	1377.98	7943.88	1.684±0.329

Table 6. Antifeedant effect of benzothiazole compounds formulated as 10% SC against 2nd and 4th instar larvae of *S. littoralis*

Compounds	Conc %	% Reduction in food consumption after 48 hrs against	
		2nd instar larvae	4th instar larvae
1	1.0	85.01	73.94
	0.5	71.35	61.52
	0.25	53.59	47.79
2	1.0	53.09	44.58
	0.5	36.34	30.46
	0.25	21.87	18.78
3	1.0	76.46	71.53
	0.5	58.09	51.33
	0.25	37.71	30.77
Chlorpyrifos 48 % EC	0.5%	84.7	73.1

All the tested compounds have antifeedant effect. on comparing between the concentrations of the formulated compounds as 10 % (SC) and the traditional products Chlorpyrifos 48 % EC it was observed that, formulation (1) gave the highest antifeedant value against both 2nd and 4th instar larvae of *S. littoralis* better than the Chlorpyrifos 48 % EC at 0.5 % followed by Formulation (3) and Formulation (2) which showed the lowest efficacy

SEMI-FIELD EXPERIMENT

Latent and developmental effect of locally formulated benzothiazole compounds were evaluated against 2nd instar larvae of *S. littoralis*. Results obtained in Table (7) indicated that the initial and the latent effect, pupation and adult emergency on the 2nd instar larvae of *S. littoralis* increased as both concentration and period after application increased. Formulation (1) with high tested

concentration gave the highest initial and latent effect on 2nd instar larvae and the lowest percentage of pupation so prevent adult emergency 100 %, comparing with other tested formulated compounds, nearly equal efficacy with the Chlorpyrifos at 0.5 %, followed by formulation (3) and formulation (2).

Table 7. Initial and latent effect of benzothiazole compounds formulated as 10 % SC against 2nd instar larvae of *S. littoralis*.

Compounds	Conc. %	% Mortality After (2days)	% Mortality up to pupal stage (10 days)	Developmental effect	
				%Pupation	% Moth emergency
1	1.0	80.7	89.3	10.7	0
	0.5	63.8	68.5	31.5	19.7
	0.25	25.6	32.4	67.6	49.4
2	1.0	36.8	43.6	56.4	36.6
	0.5	24.7	29.0	71.0	49.3
	0.25	7.4	13.8	86.2	68.4
3	1.0	45.7	59.3	40.7	30.3
	0.5	29.6	46.7	53.3	42.4
	0.25	13.7	25.4	74.6	61.7
Chlorpyrifos 48 % EC	0.5 %	81.6	100	0	0

Initial and latent effect of synthesized and formulated benzothiazole compounds were also assessed on the 4th instar larvae of *S. littoralis* also the % of pupation and adult emergency. Results obtained in Table (8) indicated that 4th instar larva of *S. littoralis* was more tolerant than 2nd instar larvae. Where the initial and latent effect value, the percent of pupation and adult of emergency with the 4th instar larvae were lower than with 2nd instar larvae at the same concentrations and conditions, where the percent of pupation was 19.6 % and the adult emergency was 10.4 % with the high concentration of formulation (1), these values were comparable to insecticide Chlorpyrifos at 0.5 % and also the initial and latent effect.

Table 8. Initial and latent effect of benzothiazole derivatives formulated as 10 % SC against 4th instar larvae of *S. littoralis*.

Compounds	Conc. %	% Mortality After (2days)	% Mortality up to pupal stage (10 days)	Developmental effect	
				%Pupation	% Moth emergency
1	1.0	72.1	80.4	19.6	10.4
	0.5	51.8	69.6	30.4	21.6
	0.25	20.7	29.5	70.5	58.3
2	1.0	25.4	33.6	66.4	45.4
	0.5	18.3	20.7	79.3	66.3
	0.25	6.8	9.3	90.7	76.2
3	1.0	40.3	51.2	48.8	36.5
	0.5	25.7	42.3	57.7	45.8
	0.25	10.5	16.7	83.3	72.4
Chlorpyrifos 48 % EC	0.5%	70.8	78.2	21.8	10.7

Generally, as shown in both Tables (7 and 8) it could be said that formulation (1) at concentration 1 % gave already the same effect of conventional insecticide, chlorpyrifos 48 % EC at 0.5%.

CONCLUSION

Benzothiazole derivatives were synthetized according to methods mentioned before, its physico-chemical properties was assessed to determine the suitable formulation form and prepared as suspension concentrate 10 %, and passed successfully all recified testes for this type of formulation. The biological efficacy of formulated benzothiazole compounds were done against 2nd and 4th instar larvae of *S. littoralis*.

1-under laboratory conditions: Toxicity of the formulated compounds were evaluated where formulation (1) showed the highest efficacy compound against the 2nd and the 4th instar larvae. Also the antifeedant effect was determined and all the tested compounds showed antifeedant effect but at variable level, where Formulation (1) gave the best effect against 2nd and 4th instar larvae, followed by formulation (3) and formulation (2).

2- Semi field experiment was done by spraying cotton plants to determine the initial and the latent effect compared with the recommended insecticide Chlorpyrifos 48 % EC, 0.5%. The results reported that formulation (1) gave the highest effect on 2nd and 4th instar larvae of *S. littorals* companied by clear in the initial and residual effect values, percentage of pupation and completely prevent of adult emergency in case of 2nd instar larvae, however the 4th instar larvae the percent of pupation was 19.6 % and adult emergency was 10.4 %, followed by formulation (3) and formulation (2). The results of formulation (1) at concentration 1 % is already as the same of recommended insecticide Chlorpyrifos 48 % EC, 0.5% therefore it could be recommended of using this safe and locally synthetized and formulated compounds of those hazard conventional insecticides in controlling cotton leafworm in different crops after completing its assessement under open field conditions.

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دراسة السمية لثلاث مركبات من البنزوثيازول مجهزة علي صورة ١٠% مركز قابل للتعلق فى الماء على دودة ورق القطن

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تم تخليق ثلاث مشتقات مختلفة لمركب البنزوثيازول بالطرق الكيميائية المعروفة, وتم دراسة الخواص الفيزيائية والكيميائية لهذة المركبات لمعرفة نوع المستحضر المناسب لها ومنها تم تجهيز هذة المركبات فى صورة مركز قابل للتعلق فى الماء تركيز ١٠ % . تم دراسة التاثير السام للمستحضرات علي العمر اليرقي الثانى والرابع لدودة ورق القطن تحت ظروف المعمل حيث اظهرت النتائج ان المستحضر الاول كان اكثر فاعلية على العمر اليرقى الثانى والرابع يلية المستحضر الثالث ثم الثانى طبقا لقيم التركيز النصفى المميت والتى كانت فى حالة العمر اليرقى الثانى كالتالى ١٦٥٧,٤٠ , ١٢٢١,٣٠ و٤٢٣,١٢ جزء فى المليون على التوالى أما فى حالة العمر اليرقى الرابع كانت كالتالى ٢٥٢٢,٣٠ , ١٣٧٧,٩٨ و ٥٤٧,٤٨ جزء فى المليون على التوالى. كذلك تم دراسة التاثير المانع للتغذية للمستحضرات الثلاث على العمر اليرقى الثانى والرابع لدودة ورق القطن مقارنة بمبيد الكلوربيريفوس (الدروسبان) أوضحت النتائج أن المستحضر الاول كان أكثر المستحضرات فاعلية وكان تأثيرة مساويا تقريبا مع المبيد التجارى دروسبان يلية المستحضر الثالث ثم الثاني. أيضا تم دراسة تاثير المستحضرات الثلاث على مراحل تطور الحشرة و أظهرت النتائج ان المستحضر الاول أعطى أعلى تأثير سام سبب موت اليرقات قبل الوصول لمرحلة التعذير , وكذلك اليرقات التى وصلت مرحلة التعذير فشلت في الوصول لمرحلة الحشرة الكاملة وخروج الفراشات.