

Original Research Article
**Impact of the application of Sulphur formulation
on the abundance and activity of insect
predators naturally existing in Marrow fields**

ABSTRACT

Aims: This work aims to explore the side effects of sulphur formulation (Sorell-98%) on two important beneficial insects to give the basis of sulphur impacts on them.

Study design: Evaluation of the effects of sulphur formulation on some predators naturally occurred in open fields.

Place and Duration of Study: Field experiment was carried out in Giza Governorate, Egypt, season 2016/2017.

Methodology: Sulphur formulation was applied to protect vegetable crops against some plant diseases and piercing sucking pests (aphis, whiteflies, thrips & mites). The experiment was undertaken in marrow fields (*Cucurbita pepo* L.). Two similar rectangle areas each area about 700m² were chosen. The first area was divided longwise into 2 halves, each half divided horizontally into 4 plots; where all agricultural practices were carried out as usual. Sorell-98% was applied at recommended rate 30 days post plantation. Similar plots at the 2nd area (check area) was chosen far enough (350 meters) from the treated one and similarly prepared as the treated one (but, without sulphur application). The average number of each natural enemy (*Coccinella undecimpunctata* & *Chrysoperla carnea*) per time interval and percent of increase and/or decrease in the population density were calculated. Fifty plants were inspected/plot/time interval in treated and untreated areas.

Results: Findings revealed that the least number of the prevailing predators' adults was recorded at the treated plots 2 hours post treatment. In addition, the mean number of the recorded adults occurred in the untreated plots of the 1st area was increased significantly than that of the untreated 2nd area at 12, 48 hours and after one week post treatment.

Conclusion: Results clarify that, the intelligent handling of sulphur formulations in order to control some pests and plant diseases with minimal or no harmful effects towards the adult predators under investigation must be taken in consideration, thus, this could be more favourable to keep the environment clean and non-polluted.

Keywords: Sulphur formulation, Sorell-98%, *Coccinella undecimpunctata*, *Chrysoperla carnea*, marrow fields.

1. INTRODUCTION

Beneficial insects in agricultural fields play an important role in natural pest control. The use of synthetic and botanical pesticides has detrimental effects to both natural enemies and pollinators. The pesticides affect the survival, reproductive capacity, changes the suitability of hosts for parasitising or predation, reduces emergence of parasitoids from sprayed host eggs and cause direct mortality [1]. This causes a serious menace to biological

control agents. When natural enemies are reduced, more serious consequences may result in pest population dynamics which include the phenomena of resurgence and eruption of secondary pests [1].

Several studies have shown the negative effect of sulphur on a wide range of natural enemies including parasitoids, predatory mites and spiders [2,3,4].

Sulphur formulation was applied in vegetable fields to control some plant diseases (mildew, powdery mildew, potato mildew, rusts...etc) and insect pests (whiteflies, thrips, aphids and mites) [5,6,7,8]. Investigations of its impact on some naturally prevailing insect predators in marrow fields *Cucurbita pepo* L. (Cucurbitales: Cucurbitaceae) were carried out by several authors [9,10,11].

This work aims to explore the side effects of sulphur formulation on two important beneficial insect predators (*Coccinella undecimpunctata* (Coleoptera: Coccinellidae) and *Chrysoperla carnea* (Neuroptera: Chrysopidae) to give the basis for research on their negative impacts on them.

2. MATERIAL AND METHODS

2.1- Material

Sulphur formulation powder (Agricultural Sorell-98%) produced by Kafr El-Zayat Pesticides & Chemicals Co., Egypt, recommended by the Ministry of Agriculture in Egypt as a protectants for vegetable crops against some plant diseases and piercing sucking pests (aphis, whiteflies, thrips & mites) was used.

2.2- Field experiment

Two similar rectangular areas which were planted with marrow plant were chosen during (2016/2017) season, each of about 700m². The first area was divided into 2 longitudinally halves, each half comprise 6 rows of plant, and divided horizontally into 4 plots each about 75m². Agricultural practices were carried out as usual including dusting by the sulphur formulation, at rate of 30 Kg/Feddan 30 days post plantation. The 2nd area was chosen far enough (350 meters) and divided typically to the 1st one, but no sulphur treatment was applied (untreated area). Records of predators' numbers were carried out for both areas at 0-time, 2, 12, 24, 48, 72 and 168 hours post treatment. The inspection was carried out at 7am. Fifty plants were inspected/plot/time interval in treated and untreated areas.

The average number of each natural enemy/50 plant/plot/time interval and percent of increase (+) or decrease (-) in the population density in the treated half and the untreated one were calculated at each time interval.

2.3- Statistical analysis

57 Analysis of variance (F-test) was applied. Duncan multiple Range test [12] was
 58 carried out to differentiate between means. Student T-test was applied between mean
 59 numbers of predators in untreated plots and untreated 2nd area. Percentage of increase or
 60 decrease was calculated using a free online tool to calculate percentages
 61 (<https://percentagecalculator.net/>).

62

63 3. RESULTS

64

65 3.1- Effect of sulphur application on *Coccinella undecimpunctata*

66

67 Data in Table (1) shows the effect of sulphur application on *C. undecimpunctata*
 68 population density in marrow cultivations.

69

70 The recorded average number of collected ladybird at 0-time and 2 hours post
 71 treatment was insignificantly different ($F_{2,9}= 1.438$ & 3.500), the same figure was observed at
 72 72 and 168 hours post treatment interval, respectively ($F_{2,9}=3.763$ & 0.775)(Table 1).

72

73 It is also clear that in the rest of inspection intervals, there was statistically significant
 74 difference between untreated plots -in one side- and treated plots in 1st area and untreated
 75 plot in the 2nd areas -on the other side-, where the average number of recorded adults was
 76 the least in the treated plots, followed by the untreated 2nd area (Table 1). It was also noticed
 77 that, after 24 hours of application, the average number of adults in treated, untreated plots
 78 and untreated plots in the 2nd area was significantly different from each other ($F_{2,9}=18.838$).
 79 After 48 hours, there was a significant difference between the average number recorded in
 80 treated plots and in both untreated plots in 1st and 2nd areas ($F_{2,9}=4.872$).

80

81 A noticeable decrease was detected 2 hours after application for all plots. The
 82 population reduction after 2 hours was (-77.97%), (-27.27%) and (-20.51%), for treated plots,
 83 untreated plots and untreated 2nd area, respectively (Table 2). There was a continuous
 84 reduction in the treated plots that reach (-11.56%) after one week of application (Table 2).
 85 The corresponding figure for untreated plots and untreated 2nd area, increased starting from
 86 12 hours (+102.27 and +30.77%), respectively, then reached about (+61%) after one week
 87 of application for both (Table 2). After 72 hours of sulphur application, the escaped adults
 88 started in returning back to the same treated plots, but still in less number compared to that
 89 recorded at 0-time (Table 1 & Fig. 1).

89

90 Table (1): Population densities of *Coccinella undecimpunctata* adults in untreated and
 91 sulphur treated marrow cultivations

92

Inspection periods (Hours)	First area		Second area	F-value (1)
	Average number of adults/50 plant/plot ± SE			
	Treated plot	Untreated plot	Untreated area	

(-) 0	14.75±1.79 aA	11.00±2.55 aB	9.75±2.10 aA	1.438 ^{NS}
(+) 2	3.25±1.90 aD	8.00±1.22 aB	7.75±1.03 aA	3.500 ^{NS}
(+) 12	6.25±1.03 bCD	22.25±2.65 aA	12.75±2.66 bA	12.790 ^{**}
(+) 24	7.00±1.08 cBCD	21.25±2.23 aA	13.25±1.25 bA	18.838 ^{**}
(+) 48	9.50±1.19 bABC	19.75±1.44 aA	16.50±3.66 abA	4.872 [*]
(+) 72	11.25±1.11 aABC	18.50±1.19 aA	15.00±2.80 aA	3.763 ^{NS}
(+) 168 (7 days)	12.75±3.75 aAB	17.75±2.90 aA	15.75±1.44 aA	0.775 ^{NS}
F-value (2)	4.381 ^{**}	6.241 ^{**}	1.936 ^{NS}	

** Highly significant * Significant NS = Insignificant (df 1=2,12) (df 2=6,28)
 In a horizontal row, means followed with the same small litter(s) are not significantly different ($P=5\%$)
 In a vertical column, means followed with the same capital litter(s) are not significantly different ($P=5\%$).

Table (2): Percentage of increase or decrease in population densities of *Coccinella undecimpunctata* adults in untreated and sulphur treated marrow cultivations

Inspection periods (Hours)	First area		Second area
	Percentage of increase (+) or decrease (-)		
	Treated plot	Untreated plot	Untreated area
(-) 0	---	---	---
(+) 2	- 77.97	- 27.27	- 20.51
(+) 12	- 57.63	+102.27	+ 30.77
(+) 24	- 52.54	+ 93.18	+ 35.90
(+) 48	- 35.59	+ 79.55	+ 69.23
(+) 72	- 23.73	+ 68.18	+ 53.85
(+) 168 (7 days)	- 11.56	+ 61.36	+ 61.54

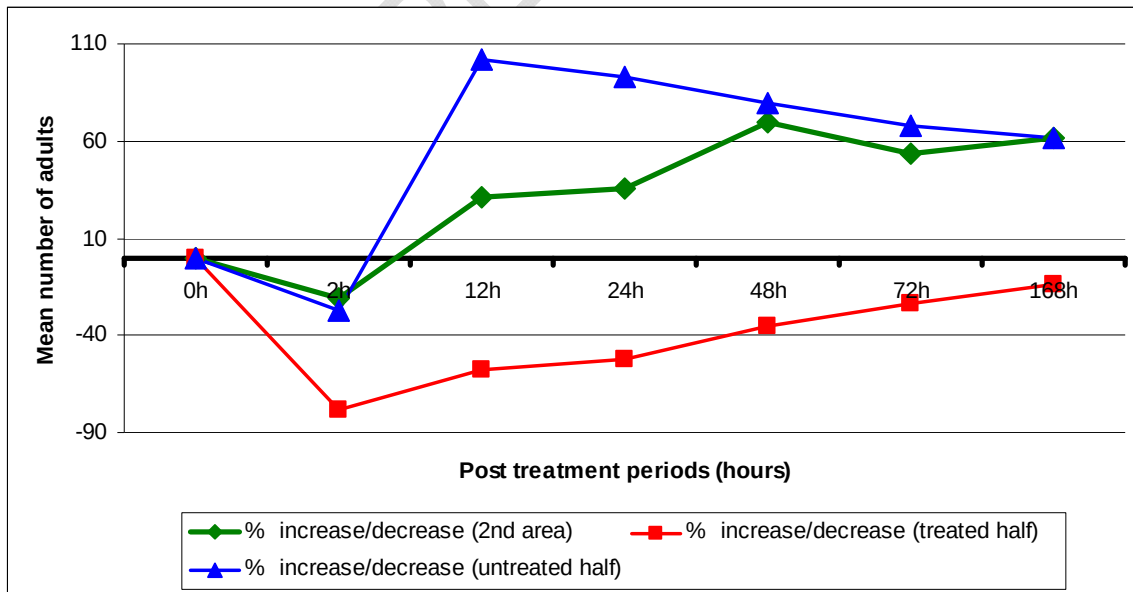


Fig. 1. Percentage of density fluctuations of *Coccinella undecimpunctata* adults in untreated and sulphur treated marrow cultivations.

3.2- Effect of sulphur dust on *Chrysoperla carnea*

On applying the same recommended rate (30 Kg/Feddan) the average density in the treated plot just before application, was 17.20 adults/50 plant/plot. The corresponding density in untreated plot, adjacent to the treated one, was 18.40 adults, while at the 2nd untreated area it was 18.40 adults; being insignificantly different (Table 3). Two hours after application, the recorded predator density averaged 9.40 adults which represents about (-45%) reduction in population (Tables 3&4). In the 2nd untreated area, the corresponding figure was (+64%) increase in population number. Thereafter, densities in treated plots showed reduction in population that ranged between (-54.65 and -8.14%) within 2 days post application and continuously to reach about (-35%) reduction after one week. This was contrary to what occurred in untreated plots adjacent to treated one as the average densities of the predator was (+18.48 to +65.22%) increase in the population density within the same period, respectively, then reached (+60.87%) after one week (Table 4 & Fig. 2). On the other hand, the population density of *Chrysoperla* adults in the untreated 2nd area recorded an increase that ranged between (+78.26 to +4.35%) within the period starting from 2 until 48 hours post treatment, then decreased gradually to reach (-35.87%) after one week (Table 4 & Fig. 2).

Comparing the density of the predator in treated and untreated plots and untreated in the 2nd area showed that, only at 0-time there was insignificant differences between them ($F_{2,12}=0.404$)(Table 3). While in the rest of all inspection periods, there were statistically significant differences detected at 2, 12 & 48 hours after application between the treated plots, untreated plots, and the second untreated area. At 72 hours and one week of application, a significant difference was detected between the untreated plots and either of treated plots and untreated area (Table 3), where the average number of recorded adults in untreated plots was more than that recorded in the others.

It is worth to notice that, when the treated plots were sprayed most of the prevailing adults migrated to the untreated adjacent plots escaping from the dust to other clean plants (in untreated plots and/or other surrounding plants), where the mean number of the recorded adults was more or less as the same of those recorded in the untreated 2nd area (Table 3 & Fig. 2).

Table (3): Population densities of *Chrysoperla carnea* adults in untreated and sulphur treated marrow cultivations

Inspection periods (Hours)	First area		Second area	F-value (1)
	Average number of adults/50 plant/plot ± SE			
	Treated plot	Untreated plot	Untreated area	

(-) 0	17.20±0.97 aA	18.40±1.22 aD	18.40±1.17 aB	0.404 ^{NS}
(+) 2	9.40±1.21 cDE	21.80±0.86 bC	30.20±0.86 aA	111.728**
(+) 12	7.80±0.58 cE	27.20±0.66 bB	30.80±0.86 aA	302.079**
(+) 24	12.20±1.07 bC	30.40±0.93 aA	32.80±0.86 aA	138.934**
(+) 48	15.80±0.86 cAB	28.20±0.86 aAB	19.20±0.86 bB	55.477**
(+) 72	13.40±0.51 bBC	26.80±0.86 aB	15.60±1.03 bC	75.204**
(+) 168 (7 days)	11.20±0.49 bCD	29.60±0.93 aAB	11.80±1.07 bD	146.375**
F-value (2)	15.320 **	23.709 **	75.838 **	

** Highly significant * Significant NS = Insignificant (df 1=2,12) (df 2=6,28)
 In a horizontal row, means followed with the same small litter(s) are not significantly different ($P=5\%$)
 In a vertical column, means followed with the same capital litter(s) are not significantly different ($P=5\%$)

Table (4): Percentage of increase or decrease in population densities of *Chrysoperla carnea* adults in treated and untreated sulphur marrow cultivations

Inspection periods (Hours)	First area		Second area
	Percentage of increase (+) or decrease (-)		
	Treated plot	Untreated plot	Untreated area
(-) 0	---	---	---
(+) 2	- 45.35	+ 18.48	+ 64.13
(+) 12	- 54.65	+ 47.83	+ 67.39
(+) 24	- 29.07	+ 65.22	+ 78.26
(+) 48	- 8.14	+ 53.26	+ 4.35
(+) 72	- 23.84	+ 45.65	- 15.22
(+) 168 (7 days)	- 34.88	+ 60.87	- 35.87

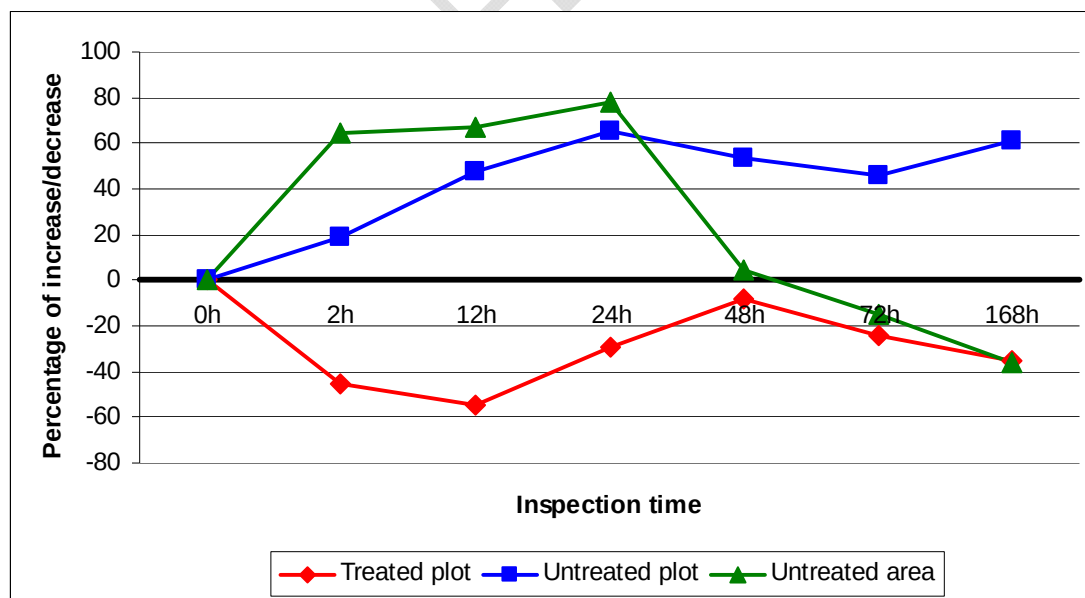


Fig. 2. Percentage of density fluctuations of *Chrysoperla carnea* adults in untreated and sulphur treated marrow cultivations.

4. DISCUSSION

4.1- In case of *Coccinella* experiments

The present results were in accordance with those reported by [7] who found that, sulphur treatment applied at the middle of the flowering stage had a small effect on *Rachiplusia nu* larvae, but had a repellent action against nabids and coccinellids. Results also matched with those reported on *Nesidiocoris tenuis* a predator of *Tuta absoluta* in tomato plantation treated with sulphur formulations [13]. Dustable sulphur, applied weekly on tomato seedlings infested with *T. absoluta* in greenhouse, significantly reduced the infestation levels and had a repellent effect on female predator. In the side effect experiment conducted on *N. tenuis*, it was found that dustable sulphur was moderately harmful as a fresh residue and slightly harmful as a 7-day-old residue; but no effects were recorded when exposing the predator to 14-day-old sulphur residues. The obtained results were partially matched with that reported in their work on the predator *Orius laevigatus*, where they found that wettable sulfur was harmless (Ex < 30%, IOBC class 1) [14].

In addition, the obtained results were in accordance with those of who stated that, powder sulphur (a.i. 95%) was harmless to the coccinellid predator *Cryptolaemus montrouzieri* [15].

However, the present results were in contrast with those reported to estimate the effects of lime sulphur on predaceous mite, *Typhlodromus pyri*, in field experiments on apple, vine and plums to control some pests (*Panonychus ulmi*, *Tetranychus urticae* and thrips) [16]. It was reported that the significant reduction of *T. pyri* and larvae of predaceous gall midges, the complete loss of mite-diversity and the possible enhancement of *P. ulmi* and other harmful mites (i.e. *Colomerus vitis*) were clear disadvantages of using lime sulphur.

Present results were in conflict with those found when conducted their studies to detect interference in the field, population density of naturally occurring mycophagous coccinellid, *Psyllobora vigintimaculata*, where vineyard density of *P. vigintimaculata* was reduced in vines receiving applications of sulphur [17]. They also mentioned that Wettable sulfur was toxic to adults and larvae of coccinellids, *P. vigintimaculata* in the laboratory, resulting in complete cohort mortality 24 hours after application. Ndakidemi et al. [18] reported that some examined pesticides had lethal effects on predacious ladybird beetles after application.

4.2- In case of *Chrysoperla* experiments

The obtained results were in accordance with those reported by Moura et al. [19,20] in their research on *Chrysoperla externa*. They reported that, abamectin, sulfur and trichlorfon were harmless to adults, while methidathion, carbaryl and fenitrothion were harmful, according to the classification of IOBC. In field studies to control powder mildew and

192 mites in Australia, results indicated that sulphur application persists on leaves more than 40
193 days post application [21]. This finding matches, more or less, with the our present results
194 where the adult predators were kept away from the treated marrow plants for more than 10
195 days.

196 Six pesticides were examined on eggs and immature stages of *Chrysoperla externa*
197 under laboratory conditions [22]; they reported that Sulfur (4.00 g/L), copper oxychloride
198 (5.00 g/L) and azociclotin (0.31 g/L) were classified as harmless (Ex < 30%, IOBC class 1),
199 selective to eggs of *C. externa* and did not affect the subsequent developmental stages. In
200 addition, they mentioned that their effects on the adult stage mortality was insignificant
201 compared to the control. These results were matched with the present results concerning the
202 non-toxicity of sulphur to the tested natural enemies.

203

204 5. CONCLUSION

205 The obtained results clarify that using sulphur formulation in marrow fields in order to
206 control some sucking insect pests and plant diseases, case minimal or no harm to the adult
207 predators. Nevertheless, other researchers reported diverse results, i.e., some of them found
208 that sulphur formulations had harmful impacts. In order to keep the environment more safe
209 and less polluted it is advised to carry out researches that are more intelligent before using
210 sulphur formulations in the open fields or under greenhouse conditions.

211

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218

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220 manuscript. Sahira Marie and Ola El-Fandary, collected the publications needed, shared in
221 constructing the tables & figures, shared in interpreting the results, in editing manuscript and
222 revised the final form. Nagy Farag and Atef Abd El-Rahman sharing in executing field
223 experiments, collecting specimens and gathering data and publications needed. Mohamed
224 Gesraha, carried out the statistical analysis, interpreted the analyzed data and obtained results,
225 constructed the tables & figures and edited the manuscript. All authors read and approved the
226 final manuscript.

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