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EVALUATING THE EFFECTIVENESS OF DIFFERENT CHEMICALS IN TREATING NOSEMA SSP HONEYBEE (*APIS MELLIFERA*) COLONIES UNDER ASWAN REGION CONDITIONS

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ABSTRACT

This research was performed during the two seasons of 2020-2021 to evaluate the effectiveness of four different treatments in controlling *nosema* spp. in honeybee colonies located in Kom-Ombo city – Aswan governorate-Egypt. These treatments managed to reduce infection in both seasons as following: Silver nano-particles gave best results at 25ppm that reduced mortality by 99.21% -98.48% and reduced spores by 100% - 100%in the 1stand 2nd seasons, respectively, followed by Sodium chlorite's best results were at 10ppm where mortality was reduced by 90.20%- 92.59% while spores reduced by 99.96%- 94.34% in the 1stand 2nd seasons, respectively. Calcium hypochlorite's best results were at 100ppm and mortality was reduced by 99.00% - 99.08% while the spore count was reduced by 100% - 100% in the 1stand 2nd seasons, respectively. The aqueous Idoine (Lugol's iodine) best results at 100ppm and reduced mortality by 82.17% -86.30% and reduced spores by 97.57% - 90.39% in the 1stand 2ndseasons, respectively. Although the four treatments seemingly successful intreating nosema; negative symptoms appeared in higher concentrations. At 1000ppm of Lugol's iodine and sodium chlorite caused death of colony. While the calcium hypochlorite caused queenless hive and laying workers at the same concentration. In general, the four treatments appeared to be effective and safe for bees as they managed to reduce infection with least losses of workers and restored the colony activities.

INTRODUCTION

Nosema disease, or nosemosis of honey bees has been regarded as a serious obstacle for profitable beekeeping in temperate climates **(Fries,1993).** Two microsporidia species have been shown to infect *Apis mellifera, Nosema apis* and *Nosema ceranae*. The honeybee immune response is significantly suppressed by *N. ceranae* infection, although this effect was not observed following infection with *Nosema apis*. Immune suppression would also increase susceptibility to other bee pathogens and senescence **(Khan, *etal*.,2019)**. The disease approaches honeybees by ingesting Nosema spores through food sharing or by ingesting fecal waste from an infected nest mate. When ingested, the *Nosema* replicates inside of midgut's cells and consume nutrition from the honey bee. Eventually the spores replicate so much that they rupture the midgut cell membrane. This release more spores into the honey bee’s midgut and induce infection to be transmitted to other bees **(Mortensen, *et al*.,2020)**. The symptoms of infection by *N.apis* are easily recognized by the large numbers of dead bees within the colony and diarrhea stains at entrance of hives indicating gastrointestinal disorders**(Bourgeois, 2010 and Abdel-Baki, 2016)**. Nevertheless, there's no effective treatments available for *Nosema* disease and the disease mechanisms underlying the pathogenic effects of *N. ceranae* infection in bees are poorly understood **(Rodriguez-Garcia, *et al*., 2021)**. Other pathogens may be present as infection by *N. apis* is often accompanied with viral pathogens such as: black queen cell virus (BQCV), bee virus Y (YV) and filamentous virus (FV). Particularly, *N.apis* infection accompanied with BQCV virus causes harm more than nosema alone. Also, they appear to be frequent in the company of the protozoan *Malpighamoeba mellificae*, which maybe the reason for the aforementioned dysentery **(Schmid-Hempel, *et al.*,1998; Fries, et al., 2013 and Galajda, *et al*., 2021).** Beekeepers may control *nosema* infection in the apiary by following basic procedures like cleaning tools & boxes, migrating or isolating the infected colonies from the healthy ones, also the periodic inspection of colonies especially packaged bees–for early detection-is necessary before placing them in hives. Although these procedures are useful in controlling *nosema* spp.; infection usually spread in all of the apiary as nosema is a highly transferrable disease through food sharing. Many researches and studies have shown the effectiveness of various kinds of treatments ranging from natural such as herb extracts and selective diets, to chemicals that are also varied in their mechanisms and effectiveness.

- The aim of this research is evaluating the effectiveness and safety of different chemical treatments in controlling nosema disease in honeybees (*apis mellifera*).

MATERIALS AND METHODS

This research was conducted during two seasons of 2020 and 2021. thirty nine colonies of first hybrid carniolan bees infected by *N.*Spp. were selected to evaluate the effectiveness of four treatments in controlling *nosema* spp. under Aswan governorate conditions. An apiary with total of 155 colonies had been selected to perform the study; located in Abbassia village- Kom Ombo city in Aswan governorate (about 45 km north Aswan).The apiary contained 155 colonies in Langstroth hives. Colonies were naturally infected by nosema and treatments were applied once every 3days. Samples were collected weekly and all of the required tests and diagnostics were performed in the Agricultural Research Center in Aswan.

Symptoms and diagnosis:

Observations such as crawling bees can be noticed in the first three days of heavy honey flow. Apparently; workers are too weak to handle heavy loads of nectar. If the gut is removed by pulling the last abdominal segment, the brown feces laden hindgut is seen first and then the midgut. In healthy bees, the midgut is amber and translucent; while in nosema-infected bee the midgut is often swollen and milky **(Moeller,1978; and Fries, *et al*2013).**

Sampling

Samples were collected weekly, each sample contains 5 adult bees taken from the entrance of each infected colony. Samples are taken for each concentration of each treatment (36 samples treated; and 3 control (sugar syrup only). All samples are transferred in ice tank to the laboratory and kept frozen under (-5 C). Treatments were given by directly spraying/dropping them on bees/combs insuring quick consumption. That is due to the short lifespan of some active ingredients. Spraying/dropping the treatment also helps disinfecting surfaces that often contain nosema spores such as: combs, brood, or honey cells and also the infected workers.

Laboratory tests

Every sample represents an infected colony and it is prepared as 5ml in volume for the whole sample (5bees), distilled water is added to complete to 5ml. Based on the content of the bees' intestine with large pollen amounts; dilution may be necessary to be able to see the spores under light microscope. Then grinding sampled bees and their abdomen or ventriculus separately in water by a mortar and a pestle.

Spores count was established by using a counting chamber (also called a hemacytometer) **(Human, *et al.*, 2013)**. 400XMicroscope a compound microscope with magnification of 100x and 400x was used. Spore count was calculated as followed: The raw spore count in 5 blocks *4million /80=number of spores. **(Mortensen, *et al*., 2020).**The two microsporidian differs their shape, where N. *cerana* is elongated and thinner than N. *apis*. That is often oval **(Friesetal,2013**)

Nosema Control:

All treatments were given to bees in three concentrations in the feeding solution (1:1) Water:

Sugar(150ml/colony)and they are as following:

1. Silver nano particles(SNP):

Nano silver and its solutions, which exhibit bactericidal, virucidal, and fungicidal effect. Silver blocks the respiratory chain and, in fungi, inhibits water binding during the developmental cycle **(Roman,2009; Allahverdiyev,*et al*., 2011; Li, *et al*., 2008; Borsuk, *et al*., 2013; and Laroo,2013)**. Silver Nano-Particles are generated by the electrolysis of silver element in distilled water. A scientific grade silver with 99.99% purity is used, production method and adjusting concentrations are according to **(Jefferson, 2003; Laroo, 2013 and Ravendranand, *et al*., 2021)**. In the study the concentrations of SNP were 5ppm, 10ppm and 25ppm in the feeding sucrose solution.

2. Lugol's iodine (KI3):

Also known as aqueous iodine or strong iodine solution **(Kaiho, 2014)**. Lugol's solution (LS)was developed 1829 by the French physician Jean Guillaume August Lugol, initially as a cure for tuberculosis. It is a solution of 5% elemental iodine and 10% potassium iodide together with distilled water. It has been used as a disinfectant, a reagent for starch detection in organic compounds, in histologic preparations, in dental procedures and in diagnosis of cervical cell alterations, the Schiller´s test **(Calissendorff, and Falhammar, 2017)**. Lugol's iodine is highly soluble in water. Like other Iodide salts, KI forms I_3^- when combined with elemental iodine. In the study, Lugol's iodine was used in concentrations 10ppm(0.0192 mg/L),100ppm (0.192mg/L) and 1000ppm (1.92mg/L) in the feeding solution according to **(Mundinger, and Slate,1952).**

3. Sodium chlorite(NaClO2):

Sodium chlorite of Acidified sodium chlorite (ASC), an antimicrobial agent, is prepared by mixing a sodium chlorite (NaClO2) solution with a generally recognized as safe organic acid. This chemical reaction produced active chlorine dioxide showing bactericidal activity in combination with acidity. The U.S. Food and Drug Administration has approved ASC for use in poultry, red meat, comminuted meat products, and processed fruits and vegetables to reduce bacterial contamination **(Anonymous, 1999 and Inatsu, *et al*., 2004)**. Additionally, ClO2 gas has potent virucidal properties *in vivo*, specifically against influenza A viral infection in mice. Mouth rinse studies of ClO2 have demonstrated antibacterial and antifungal properties when used in people. The solutions ranged from 0.003% to0.016% (wt/vol%)ClO2. **(Grootveld, *et al.* 2001; Mohammad, *et al.* 2004 and Ogata, *et al*.2008).**

Sodium chlorite is activated by reacting with food grade acids (e.g. HCL, Acetic acid, citric acid, etc) to release the active ingredient (Clo_2). The reaction results a concentrated mixture that contains Chlorine Dioxide ClO_2 (the therapeutic agent), Sodium Citrate and Sodium Chloride**.** Sodium chlorite is diluted by the feeding solution (1:1sugar : water) to three concentrations with content of10ppm (o.o357 Mg/L),100ppm(o.357 Mg/L) and 1000ppm(3.57Mg/L), according to **(James, *et al.* 1972)**.

4. Calcium hypochlorite Ca(OCl)2:

Calcium hypochlorite, sometimes referred to as powder chlorine, powder bleach, or highest hypochlorite is a solid containing 65-70% available chlorine. It is often available in compressed form (e.g. tablets, briquettes) and also as granules or powder **(Connell, 2006).** In the study food grade Calcium hypochlorite (granules) was used, (1gm/L=650ppm)**.** calcium hypochlorite is given to bees in the feeding solution by dissolving the powder with the required concentration in water first, then adding sugar. The concentrations used in the study are 10ppm (0.0153g/L), 100ppm (0.153g/L) and 1000ppm (1.538g/L).

$$\text{Reduction ratio \%} = 1 - \frac{Ta\ x Cb}{TbxCa} \times 100$$

Where:

Ta is % infection by nosema after treatment.

Tb is % infection by nosema before treatment.

Ca is % infection by nosema after treatment for the control.

Cb is % infection by nosema before treatment for the control. (Henderson, and Tilton,1955)

Statistical analysis:

Analysis of variance (ANOVA) was performed for the obtained data according to test multiple groups by Waller, and Duncan,(1996)

REULTS AND DISCUSSION

1.SilverNano-Particles(SNP):

Data in tables (1)&(3) and Figs (2)&(4) showed that mortality of bees in the 1stseason at 5ppm from 136.32 to 8.66, while at 10ppm it dropped from 151.32 to 3.32; and at25ppm it dropped from 131.32 to 13.32in one week then reduced to2.66at the end of the study. Results were similar in the second season 2021 and 25ppm was most effective in reducing mortality in both seasons. Tables (2)&(4)and Figs(3)&(5)showed that infection rates at 5ppm has dropped from $7.36x10^6$ to $1.65x10^6$ spores/bee;7.25 to 1.40 $x10^6$ spores/bee. While 10ppm solution reduced infection from $8.72x10^6$to $0.51x10^6$spores/bee; and from $10.14x10^6$to$0.35x10^6$spores/bee. The 25ppm solution reduced infection rates from $8.75x10^6$to0.00 spores/bee; and from 9.28 to 0.00 spores/bee in the 1st and 2nd seasons, respectively. Although test results in the first week did not show significant reduction in the spore count; the number of dead bees had been tremendously reduced due to relief of symptoms; or death of the accompanied pathogens like viruses or bacteria, **(Bailey, *et al*., 1983; Dainat. *et al*., 2012).** reported that both microsporidia were associated with presence of the Black queen cell virus (BQCV). Silver nano particles is an antibiotic of a wide spectrum of pathogens including viruses. That comes in agreement with **(Laroo, 2013),** who reported that a sample of “Silver Water Solution” has successfully demonstrated antimicrobial activity against *Enterococcus faecalis* and *Candida albicans* by achieving greater than 5.2 log reduction (or<99.99% kill) and 3.57 log reduction (or<99.99%kill), respectively. Also, **(Khan, *et al*.,2019)** stated that silver nano-particles being measured for use as an alternative control in bee hives requires significant inhibitory activity against the bee disease without nontoxic effect on adult honeybees. Silver nano particles and its solutions, which exhibit bactericidal, virucidal, and fungicidal activity. Silver blocks the respiratory chain and, in fungi, inhibits water binding during the developmental cycle **(Roman, *et al.*,2009).**Who stated that silver nano-particles are well known potent antimicrobial agents. Although significant progresses have been achieved on the elucidation of antimicrobial mechanism of silver nano particles **(Durán, *et al*., 2016).**

Although supplementation of the honeybee diet with nano silver reduces *Nosema* spp. infection, it may cause deposition of silver in the bee's organs. The accumulation of silver in the bee organs suggests that nano silver in veterinary and medical practice should be used with caution **(Borsuk, *et al*., 2013).** However, Silver nano-particles or elemental silver appears to be safe alternative treatment for nosema in honeybees. The highest concentration in the study was 25ppm which reduced infection to 0.00spores/bee, and almost there was no related mortality of workers.

Calcium hypochlorite $Ca(OCl)_2$:

Data in tables (5) & (7)and fig (6)&(8)showed that the number of dead bees at 10ppm dropped from 174.00 to 12.00; and at 100ppm 156.00 to 4.00; while 1000ppm dropped from 142.00 to 77.32 with similar results in the 2nd season. Best results was obtained at 100ppm.

Data in tables (6) & (8) and fig (7)&(9) showed the weekly average of spore count in the1st and 2nd seasons respectively as following: 10ppmhas reduced infection rates from 7.97 $X10^6$to 1.93 $X10^6$ spores/bee; and from 9.27 $X10^6$to 0.39 $x10^6$spores/bee. The 100ppm concentration reduced infection from7.79$x10^6$ to 0.00 spores/bee; and from 7.69$x10^6$to 0.00 spores/bee. While 1000ppm reduced infection rates from 7.22$x10^6$to0.00 spores/bee; and from7.94 to 0.00 spores/bee. Best results were obtained at 100ppm, while at 1000ppm the queen disappeared and laying workers occurred which can be due to toxicity of hypochlorite fume or when ingested in high concentrations. However, it showed high efficiency as a germicide; and this came in agreement with **(Wang, and Quing, 2014)** Alfalfa seeds were inoculated with human norovirus (huNoV) geno groupII (GII), murinenoro virus(MNV),Tulane virus (TV), Escherichia coli O104:H4, and Salmonella entericaserovar Agona. The seeds were treated by calcium hypochlorite (2000 ppm or 20,000 ppm with an average of free chlorine 1388±117 mg/Land 11,472±1500 mg/L, respectively, pH adjusted to 7.00); and **(Connell,2006)**, stated that contact tanks are used to provide the mixing and detention time for the chlorine to come into contact with all bacteria and other organisms in the waste water. Also, **(Connell, 2006 and Jena, *et al*. 2017)**, mentioned that hypochlorite solutions are efficient and safe disinfectants, which was recommended by both the World Health Organization and the United States Environmental Protection Administration. It has been used in the fields of disinfection, water purification, food preservation, etc. Although hypochlorite solutions are effective to efficiently act on Nosema spores; its mechanisms are poorly understood.

Lugol's solution

Data in tables (9) & (11) and fig (10) & (12) showed that the number of dead bees at 10ppm was reduced from 136.00 to 14.66; while at 100ppm from 145.22 to 66.66; while at 1000ppm death of colony occurred. Data in tables (10)&(12) and fig (11) & (13) showed that 10ppm solution has reduced infection levels from 10.13 to $2.06x10^6$ spores/bee; and from 9.40 to 1.43 $x10^6$ spores/bee in the 1^{st} and 2^{nd}seasons, respectively. While 100ppm solution reduced infection from 8.91 to $0.33x10^6$ spores/bee; and from9.44 to1.44 $x10^6$ spores/bee in the 1^{st} and2^{nd} seasons, respectively. Best results are obtained at 10ppm. Although, the spore count did not showed significant reduction in the first week; mortality levels did in the 1^{st} dose which can be referred to death of the accompanied viruses. lugol's iodine was effective in reducing the infection, however; 1000ppm solution caused death of colony in the first week. Infection by *N.apis* usually occurs accompanied by 3 viral infections, such as: black queen cell virus (BQCV), bee virus Y (YV) and filamentous virus (FV). Particularly, the combination of *N. apis* infection with BQCV virus is more harmful than the individual infections alone.Viruses are able to add the subspecies apis virulence. Also, they appear to be frequent in the company of the protozoan *Malpighamoeba mellificae*, which may be the reason for the aforementioned dysentery **(Schmid, 1998; Galajda, *et al*., 2021 and Fries, *et al.*, 2013).** Although, less reactive than chlorine, iodine was rapidly bactericidal, fungicidal, tuberculocidal, virucidal, and sporicidal **(Gottardi,1991)**. Lugol's solution or Aqueous iodine is a solution of elemental iodine, I_2,and potassium Water content of the stored sucrose solution was below that necessary for iodide, KI, in water and is mostly used as an antiseptic and disinfectant **(Kaiho,2014)**

Sodium Chlorite ($NaClO_2$):

Data in tables (13) & (15) and fig (14) & (16) showed mortality levels as following: the average number of dead bees at 10ppm was 145.32 before treatment and reduced to 36.66; and from155.32to32.66 in the 1^{st} and 2^{nd} seasons respectively. At 100ppm dropped from 177.32to 108.66; and from 156.00 to103.32, in both seasons, respectively. At 10ppm concentration is preferable where it resulted less mortality. While at 1000ppm caused death of colony.

Data in tables(14)&(16) and fig (15) & (17) showed that 10ppm has reduced infection levels from 8.56 to $0.44x10^6$ spores/bee; and from 10.14 to $0.91x10^6$spores/bee. The 100ppm solution reduced infection rates from 6.66 to 0.00; and from 8.33 to 0.00 spores/bee in the $1^{st}$and 2^{nd} seasons, respectively. Although, increasing the concentration is beneficial against nosema; 100ppm appears to be the ideal concentration as1000ppm caused death of colony as shown in tables. This comes in agreement with **(Warf,2001)** stated that Sodium chlorite ($NaClO_2$) is a an effective sanitizer for inhibiting the growth of microbes. **(Lackett, 1970)**,found that 10ppm and100ppm chlorine dioxide given to caged bees reduced their rate of mortality significantly below all other tested dosages. The bees which were fed the above dosages built honeycomb in the cages and actually stored some of the sucrose solution after dehydrating it. fermentation. All bees fed1,000 or 10,000ppm died before the experiment was concluded. The group fed 10,000ppm reached 100% mortality within seven days; 100% mortality in the 1,000ppmgroup occurred at 21 days. Acidified sodium chlorite is a highly effective antimicrobial agent that is produced by lowering the pH(2.5–3.2) of a solution of sodium chlorite($NaClO_2$; SC)with any GRAS acid.

SUMMARY

it be concluded that all of the tested treatments were affective and successfully reduced nosema symptoms in bees. Silver nano-particles reduced infection by 100% with no noticeable side effects. Sodium chlorite also reduced infection by 100%, however, there was negative side effects like high mortality of workers or death of the colony at 1000ppm, which may be due to related toxicity. Calcium hypochlorite showed similar results to sodium chlorite except it is less toxic,at1000ppm death of the queen and emergence of laying workers occurred. Lugol's iodine also showed effectiveness with 84.75% at 100ppm,also showed negative side effects similar to chlorines. Iodine did not significantly decrease spores count, however, the average of dead bees has decreased probably due to elimination of secondary infection.

Table (1): Mean of bee mortality (no. dead bees/week) of nosema infected colonies treated by silver nano particles during season 2020.

DATE	Silver nano particles (SNP)			
	5ppm	**10ppm**	**25ppm**	**Control (sugar syrup)**
Before treatment	156.32 F	151.32 FG	131.32 G	152.7 FG
28/02/2020	40.00 H	29.32 HI	13.32 HI	182.7 EF
06/03/2020	30.00 HI	12.00 HI	7.32 HI	210.7 E
13/03/2020	18.00 HI	8.66 HI	5.32 HI	260.00
20/03/2020	8.00 FGH	5.32 HI	4.00 HI	268.7 CD
27/03/2020	6.00HI	4.66 HI	2.00 I	304.00 B
03/04/2020	8.66 HI	4.66 HI	3.32 HI	293.3 BC
10/04/2020	7.33 HI	2.66 HI	4.00 HI	363.3 A
17/04/2020	8.66 HI	3.32 HI	2.66 HI	393.3 A
Mean	32.44 B	24.66 BC	19.26 C	269.9 A
Reduction %	94.46	99.14%	99.21%	

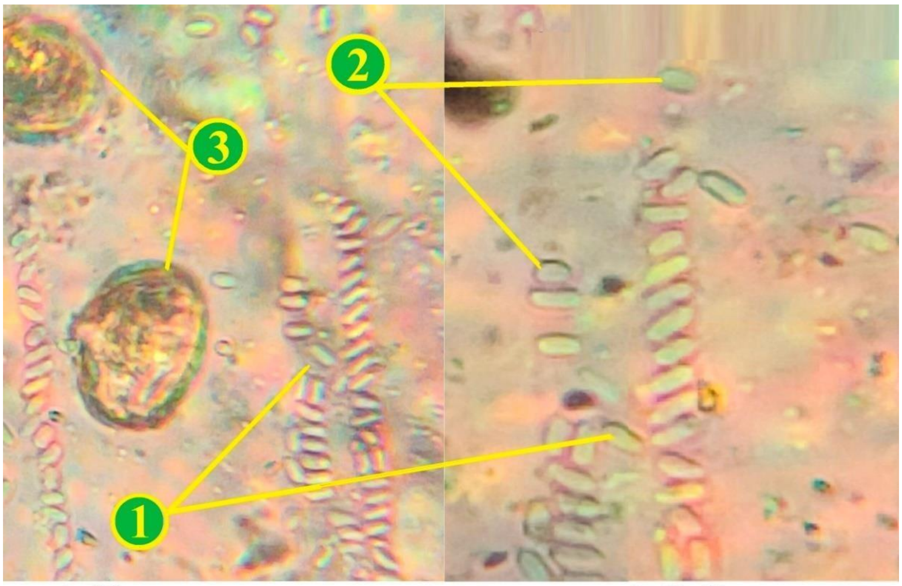

Fig (1): Nosema spores under light microscope

Table (2): Mean of spores count (no. spores x106/bee) of nosema infected colonies while treated by silver nano particle during 2020.

DATE	Silver nano-particles (SNP)			
	5ppm	**10ppm**	**25ppm**	**Control (sugar syrup)**
Before treatment	7.36FG	8.72FG	8.75GH	8.28 FG
28/02/2020	6.29GH	6.23HI	6.77KL	8.76 EFG
06/03/2020	4.81HI	4.90JK	5.42NO	9.30DEF
13/03/2020	4.30 IJ	3.69 LMN	3.20 QR	10.03 CDE
20/03/2020	3.64 JK	2.63 OP	2.31 S	10.63 BCD
27/03/2020	3.08 KL	1.92 PQ	1.52 S	11.22 ABC
03/04/2020	2.56 LM	1.41 RS	0.45 S	12.17 AB
10/04/2020	2.35 MNO	1.04 S	0.04 S	12.68 A
17/04/2020	1.65 O	0.51 S	0.00S	12.65 A
Mean	4.00 B	3.45 C	3.16 D	10.63 A
Reduction %	85.40%	96.17%	100%	

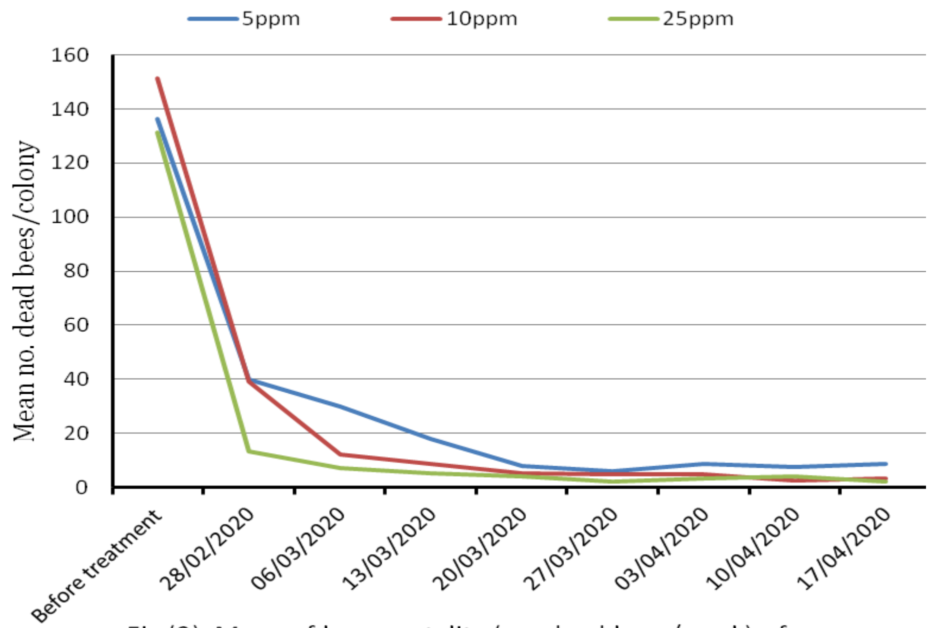

Fig (2): Mean of bee mortality (no. dead bees/week) of nosema infected colonies treated by silver nano-particles during season 2020.

Table (3): Mean of bee mortality (no. dead bees/week) of nosema infected colonies treated by silver nano-particles during season 2021.

DATE	Silver nano-particles (SNP)			
	5ppm	**10ppm**	**25ppm**	**Control (sugar syrup)**
Before treatment	157.32J	169.32 IJ	154.66 J	184.0 I
28/02/2020	40.00 KL	43.33 K	20.00 KLMN	113.33
06/03/2020	30.66 BC	23.32 CD	7.32 DEF	226.7 H
13/03/2020	30.67 KLM	23.33 KLMN	7.33 MN	268.0 G
20/03/2020	15.23CDEF	8.00 DEF	3.32 F	170.67
27/03/2020	12.00 DEF	9.32 DEF	4.00 F	193.33
03/04/2020	11.32 DEF	8.00 DEF	5.32 EF	215.67
10/04/2020	9.32 DEF	8.00 DEF	2.00 F	234.00
17/04/2020	10.00 DEF	8.00 DEF	3.32 F	261.33
Mean	34.22 B	32.52 B	23.11 C	348.6 A
Reduction %	95.53%	96.70%	98.48%	

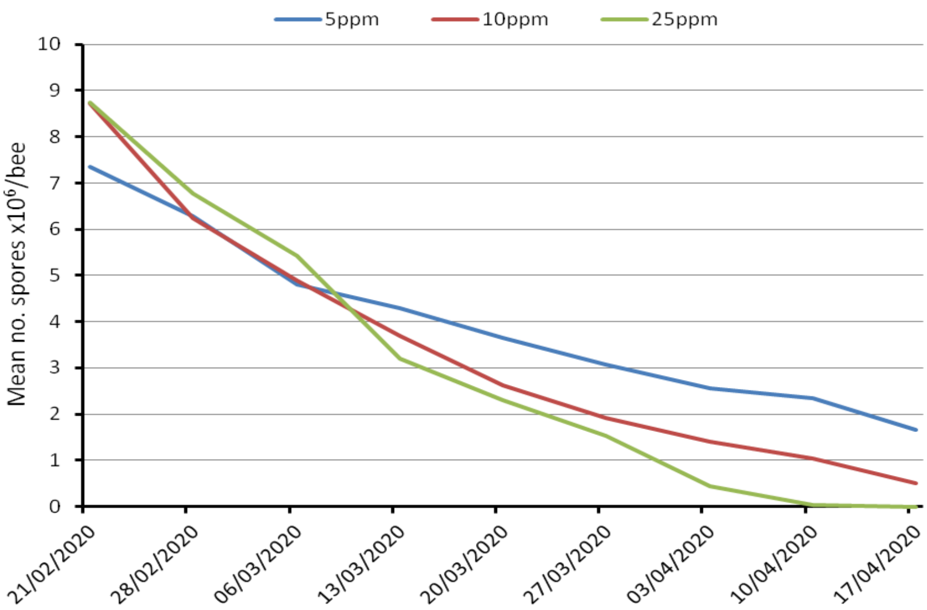

Fig (3): Mean of spores count (no. spores x10^6/bee) of nosema infected colonies while treated by silver nano particle during 2020.

Table (4): Mean of spores count (no.sporesx106/bee)of nosema infected colonies treated by silver nano particles during season 2021.

DATE	Silver nano-particles(SNP)			
	5ppm	**10ppm**	**25ppm**	**Control (sugar syrup)**
Before treatment	7.25DEFG	10.14EFGH	9.28EFG	8.41FGHI
28/02/2020	6.89GHI	8.05HIG	7.59CIJK	9.22EFG
06/03/2020	5.25IJK	6.48JK	6.77L	9.92DEF
13/03/2020	4.36KL	5.03L	5.72MN	10.49CDE
20/03/2020	3.67L	3.90M	4.19O	11.05BCD
27/03/2020	3.14M	3.38N	3.04PQ	9.82ABC
03/04/2020	2.60N	2.07O	1.73Q	12.26AB
10/04/2020	2.40O	1.06PQ	0.91Q	10.17A
17/04/2020	1.40P	0.35Q	0.00Q	13.35A
Mean	4.10B	4.49C	4.35D	10.52A
Reduction%	87.83%	97.82%	100%	

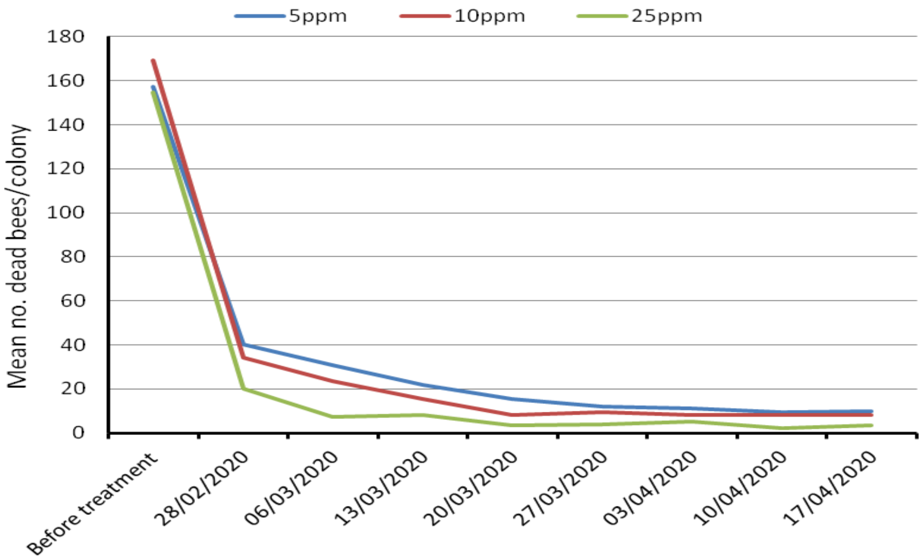

Fig (4): Mean of bee mortality (no. dead bees/week) of nosema infected colonies treated by silver nano-particles during season 2021.

Table(5): Mean of bee mortality (no. dead bees/week) of nosema infected colonies treated by Calcium hypochlorite during season2020.

DATE	Calcium hypochlorite $Ca(OCl)_2$			
	10ppm	**100ppm**	**1000ppm**	**Control (sugar syrup)**
Before treatment	174.00FG	156.00FG	142.00CGH	152.7FG
28/02/2020	113.32HI	22.66LM	99.34IJ	182.7EF
06/03/2020	85.32IJK	14.00M	85.32IJK	210.7E
13/03/2020	68.00JK	10.67M	78.67IJK	260.0D
20/03/2020	51.32KL	6.66M	85.32IJK	268.7CD
27/03/2020	34.66LM	2.66M	90.66IJ	304.4B
03/04/2020	24.66LM	2.66M	78.66IGK	293.3BC
10/04/2020	18.00LM	2.00M	76.00JK	363.3A
17/04/2020	12.00M	4.00M	77.32IJK	393.3A
Mean	64.59C	24.59D	90.37A	269.9A
Reduction%	97.32%	99.00%	78.85%	

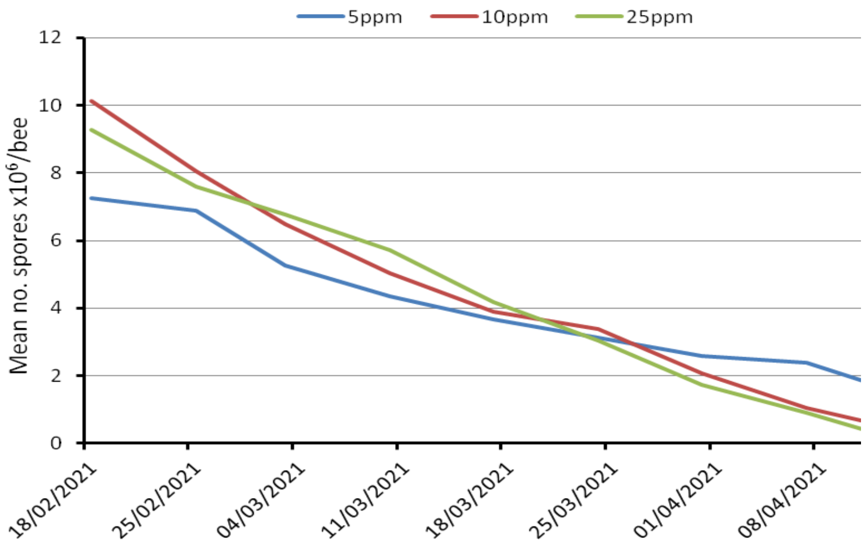

Fig (5): Mean of spores count (no. spores $x10^6$/bee) of nosema infected colonies treated by silver nano particles during season 2021.

Table (6): Mean of spores count (no. spores/bee X 106) of nosema infected colonies treated by calcium hypochlorite during season 2021.

DATE	Calcium hypochlorite $Ca(OCl)_2$			
	10ppm	100ppm	1000ppm	Control (sugar syrup)
Before treatment	7.97CDEF	7.79BCDE	7.22BCDE	8.28BCDE
28/02/2020	7.17EFGH	5.83EFGH	4.02DEFG	8.76BCDE
06/03/2020	6.07GHIJ	4.25GHIJ	2.03FGHI	9.30ABCD
13/03/2020	5.39HIJK	2.97IJKL	0.82JKLM	10.03ABC
20/03/2020	4.49IJKL	1.87KLMNO	0.15LMNOP	10.63AB
27/03/2020	3.74JKLMN	1.12MNOP	0.00NOPQR	11.22AB
03/04/2020	3.10KLMNO	0.48OPQR	0.00NOPQR	12.17A
10/04/2020	2.52GHI	0.17NOPQR	0.00NOPQR	12.68A
17/04/2020	1.93MNOPQ	0.00NOPQR	0.00NOPQR	12.65A
Mean	4.71B	2.72C	1.58C	10.63A
Reduction%	84.14%	100%	100%	

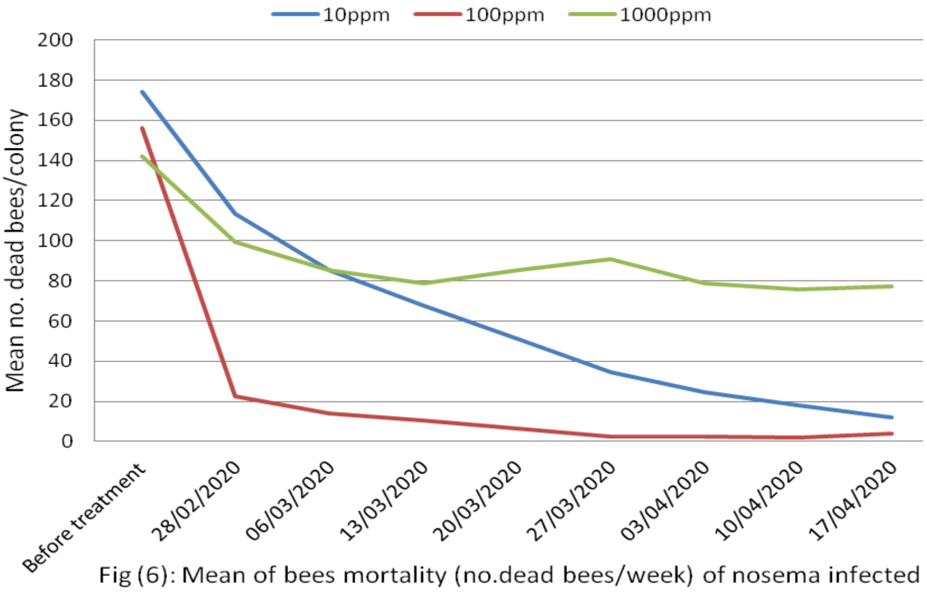

Fig (6): Mean of bees mortality (no.dead bees/week) of nosema infected colonies treated by calcium hypochlorite during season 2021.

Table(7):Mean of bee mortality (no. dead bees/week) of nosema infected colonies treatedbycalciumhypochloriteduringseason2021.

DATE	**Calcium hypochlorite $Ca(OCl)_2$**			
	10ppm	**100ppm**	**1000ppm**	**Control (sugar syrup)**
Before treatment	170.00IJ	154.00J	167.32IJ	184.0I
25/02/2021	112.00K	14.66PQ	110.66K	226.7H
03/03/2021	82.66LMN	9.32Q	98.66KL	268.0G
10/03/2021	58.00NO	5.32Q	88.66KLM	306.0F
17/03/2021	38.00OP	2.66Q	76.00EF	341.3E
24/03/2021	20.66PQ	4.00Q	62.66NO	389.7D
31/03/2021	13.32Q	6.66Q	66.00MN	431.3C
07/04/2021	12.66Q	4.66Q	60.00NO	468.0B
14/04/2021	12.00Q	4.00Q	61.32NO	522.7A
Mean	57.70C	22.81D	87.93B	348.6A
Reduction%	97.51%	99.08%	63.35%	

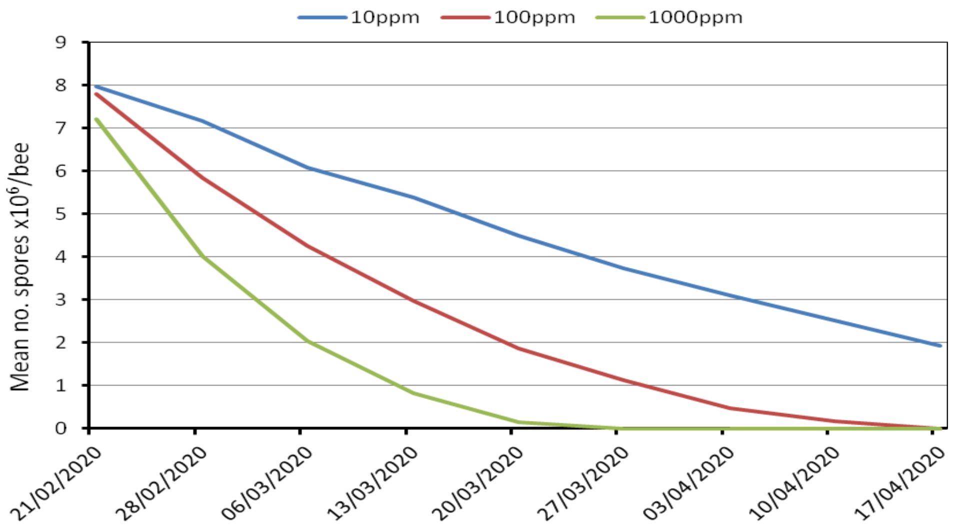

Fig (7): Mean of spores count (no. spores/bee X 10^6) of nosema infected colonies treated by calcium hypochlorite during season 2021.

Table(8):Mean of spores count (no.sporesx106/bee) of nosema infected colonies treated by calcium hypochlorite during season 2021.

DATE	Calcium hypochlorite $Ca(OCl)_2$			
	10ppm	**100ppm**	**1000ppm**	**Control (sugar syrup)**
Before treatment	9.27HIJ	7.69CDE	7.94EFG	8.41FGH
25/02/2021	8.10IJK	6.55GHI	5.55HIJ	9.22EFG
03/03/2021	7.06LM	4.82JK	3.64IJK	9.92DEF
10/03/2021	5.42MNO	3.62LMN	1.45KL	10.49CDE
17/03/2021	4.01OPQ	2.04NOPQ	0.28MNOP	11.05BCD
24/03/2021	2.77PQRS	1.16OPQR	0.00Y	9.82ABC
31/03/2021	1.80RST	0.47TUV	0.00Y	12.26AB
07/04/2021	1.12STU	0.09WX	0.00Y	10.17CDE
14/04/2021	0.39TUV	0.00Y	0.00Y	13.35A
Mean	4.43B	2.93B	2.09B	10.52A
Reduction%	97.35%	100%	100%	

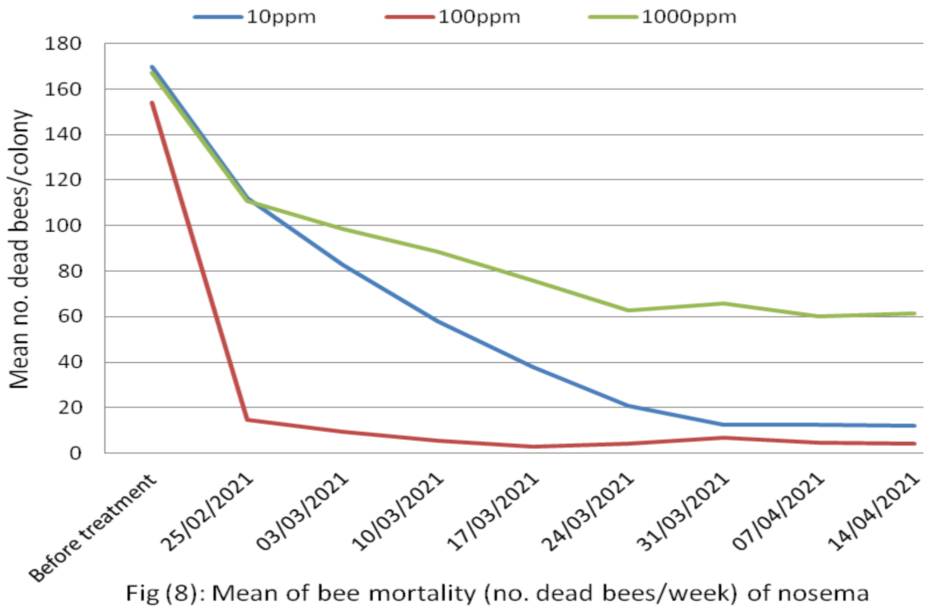

Fig (8): Mean of bee mortality (no. dead bees/week) of nosema infected colonies treated by calcium hypochlorite during season 2021.

Table (9): Mean of bee mortality (no. dead bees/colony) of nosema infected colonies treated by Lugol's iodine during season2020.

DATE	Lugol's solution(KI_3)			
	10ppm	100ppm	1000ppm	Control (sugar syrup)
Before treatment	136.00F	145.22F	140.00	152.7EF
28/02/2020	26.00HIJK	51.22GHIJ	-	182.7DE
06/03/2020	14.00IJK	39.22GHIJK	-	210.7D
13/03/2020	9.32K	54.00GHI	-	260.0C
20/03/2020	12.66JK	54.00GHI	-	268.7BC
27/03/2020	6.66K	70.00G	-	304.0B
03/04/2020	12.00JK	63.32GH	-	293.3BC
10/04/2020	12.66JK	74.00G	-	363.3A
17/04/2020	14.66IJK	66.66G	-	393.3A
Mean	27.01C	68.66B	-	269.9A
Reduction%	95.81%	82.17%		

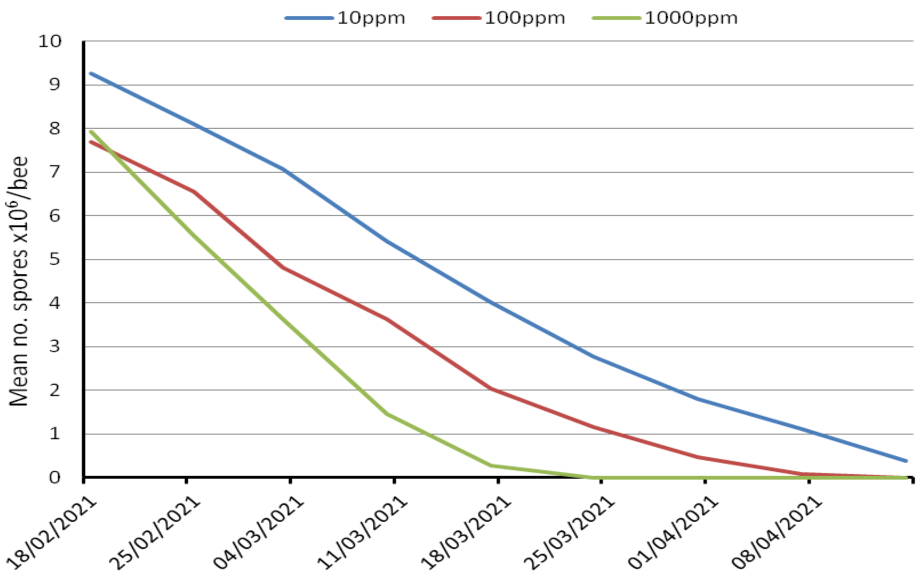

Fig (9): Mean of spores count (no. spores $x10^6$/bee) of *nosema* infected colonies treated by calcium hypochlorite during season 2021.

Table(10): Mean of bees mortality (no. dead bees/week) of nosema infected colonies treated by Lugol's solution during season2020.

DATE	Lugol's solution(KI3)			
	10ppm	**100ppm**	**1000ppm**	**Control (sugar syrup)**
Before treatment	10.13CDE	8.91EF	9.02	8.28F
28/02/2020	9.09DEF	7.79FG	-	8.76EF
06/03/2020	7.87FG	6.44H	-	9.30DEF
13/03/2020	6.88GH	5.21I	-	10.03CDE
20/03/2020	5.69HI	3.96JK	-	10.63BCD
27/03/2020	4.88IJ	2.59LM	-	11.22ABC
03/04/2020	3.78K	1.63N	-	12.17AB
10/04/2020	3.18KL	0.73O	-	12.68A
17/04/2020	2.06MN	0.33O	-	12.65A
Mean	5.95B	4.17C	-	10.63A
Reduction%	86.69%	97.57%	-	

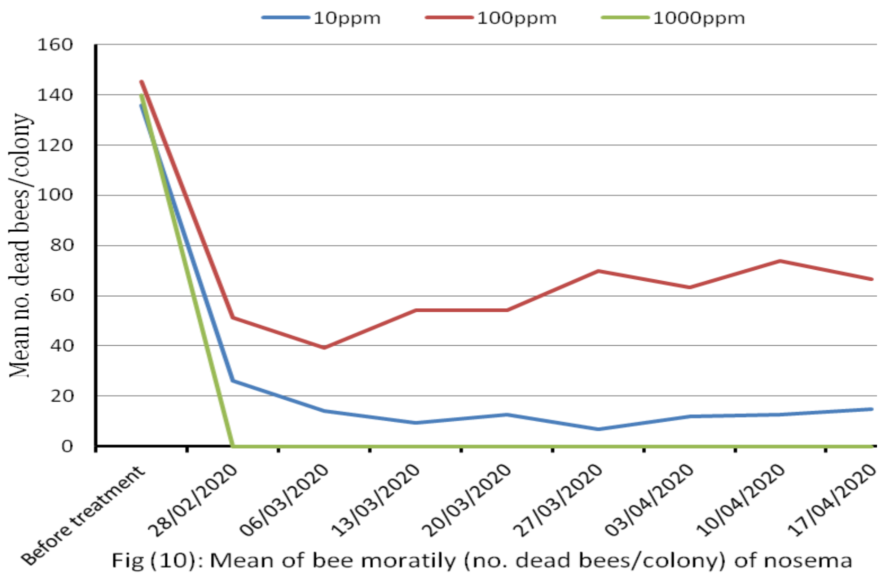

Fig (10): Mean of bee moratily (no. dead bees/colony) of nosema infected colonies treated by Lugol's iodine during season 2020.

Table (11): Mean of bees mortality (no. dead bees/week) of nosema infected colonies treated by Lugol's solution during season2021.

DATE	Lugol's solution(KI3)			
	10ppm	**100ppm**	**1000ppm**	**Control (sugar syrup)**
Before treatment	161.32J	147.32J	183	184.0I
25/02/2021	36.66KL	37.23KL	-	226.7H
03/03/2021	24.66LM	30.66LM	-	268.0G
10/03/2021	14.00M	39.32KL	-	306.0F
17/03/2021	14.00M	40.66KL	-	341.3E
24/03/2021	12.66M	43.32KL	-	389.7D
31/03/2021	13.32M	46.00KL	-	431.3C
07/04/2021	12.00M	54.00K	-	468.0B
14/04/2021	10.66M	57.32K	-	522.7A
Mean	33.26C	55.09B	-	348.6A
Reduction%	97.67%	86.30%		

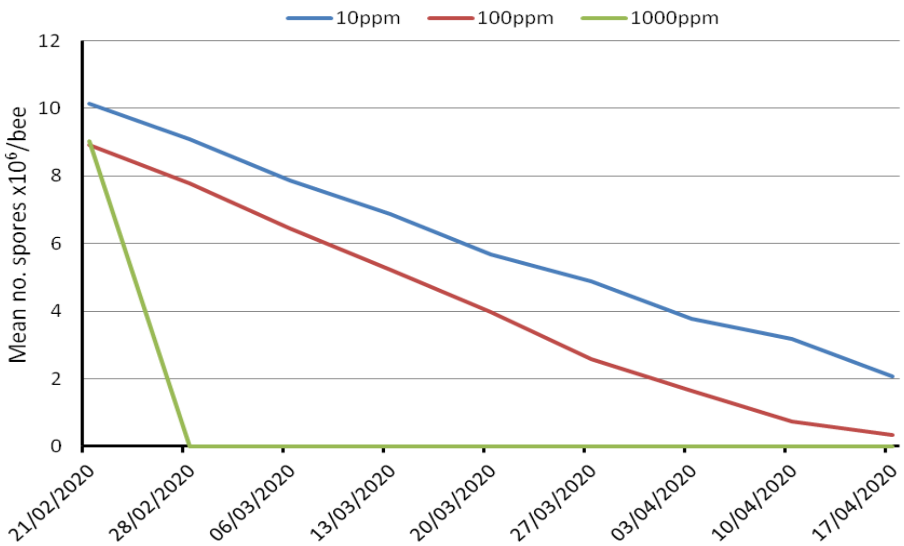

Fig (11): Mean of bees mortality (no. dead bees/week) of nosema infected colonies treated by Lugol's solution during season 2020

Table (12): Mean of spores count (no.sporesx106/bee)of nosema infected colonies treated by lugol's solution during season2021.

DATE	Lugol's solution (KI3)			
	10ppm	**100ppm**	**1000ppm**	**Control (sugar syrup)**
Before treatment	9.40EF	9.44EF	9.15	8.41FG
25/02/2021	8.38FG	8.45FG	-	9.22EF
03/03/2021	7.40GH	7.42GH	-	9.92DEF
10/03/2021	6.57HI	6.40HI	-	10.49CDE
17/03/2021	5.40IJ	5.48IJ	-	11.05BCD
24/03/2021	4.19KL	4.56JK	-	9.82ABC
31/03/2021	3.37L	3.41L	-	12.26AB
07/04/2021	2.38M	2.44M	-	10.17A
14/04/2021	1.43N	1.44N	-	13.35A
Mean	5.39B	5.44B	-	10.52A
Reduction%	90.42%	90.39%	-	

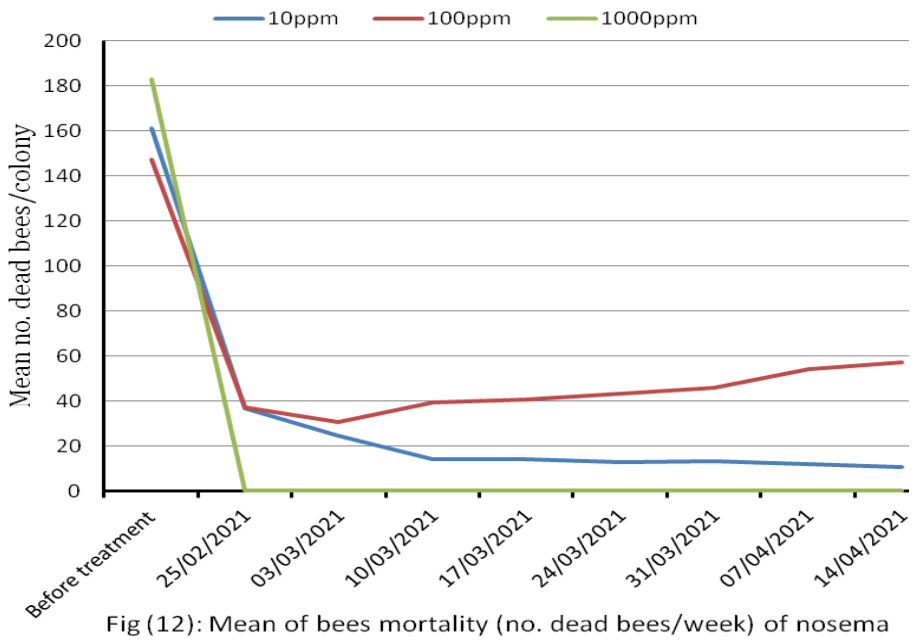

Fig (12): Mean of bees mortality (no. dead bees/week) of nosema infected colonies treated by Lugol's solution during season 2021.

Table (13):Mean of bees mortality (no. dead bees/week) of nosema infected colonies treated by sodium chlorite during season2020.

DATE	Sodium Chlorite($NaClO_2$)			
	10ppm	**100ppm**	**1000ppm**	**Control (sugar syrup)**
Before treatment	145.32FG	177.32DEF	193.80	152.7EF
28/02/2020	74.66HI	100.66H	-	182.7DE
06/03/2020	59.32I	107.32GH	-	210.7GH
13/03/2020	54.00I	111.32GH	-	260.0C
20/03/2020	54.66I	112.00GH	-	268.7BC
27/03/2020	49.32I	110.00GH	-	304.0B
03/04/2020	44.66I	110.66GH	-	293.3BC
10/04/2020	44.66I	109.32GH	-	363.3A
17/04/2020	36.66I	108.66GH	-	393.3A
Mean	62.58C	116.36B	-	269.9A
Reduction%	90.20%	76.20%	-	

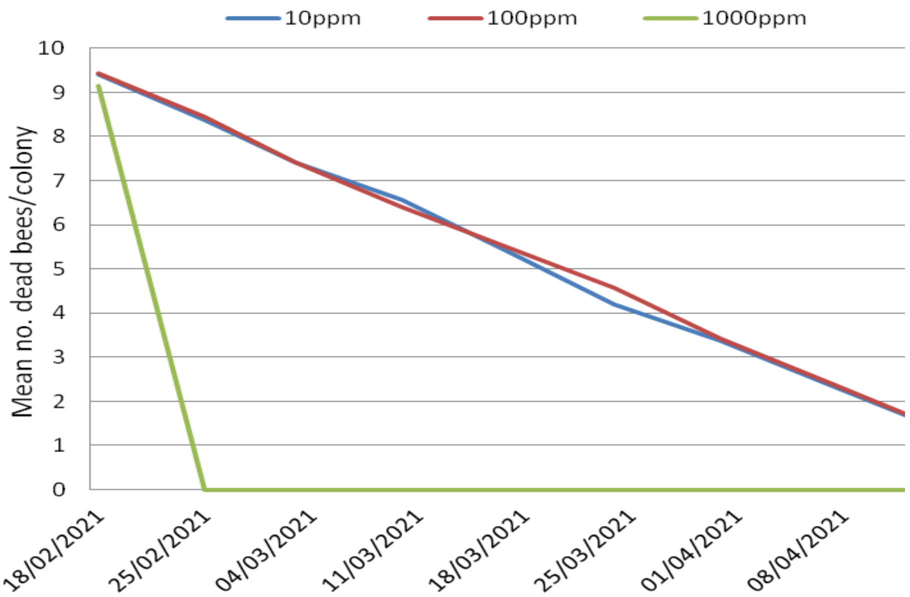

Fig (13): Mean of spores count (no. spores x10^6/bee) of nosema infected colonies treated by lugol's solution during season 2021.

Table(14): Mean of spores count (no.sporesx106/bee)of nosema infected colonies treated by sodium chlorite during season2020.

DATE	Sodium Chlorite ($NaClO_2$)			
	10ppm	**100ppm**	**1000ppm**	**Control (sugar syrup)**
Before treatment	8.56CDE	6.66EF	9.19	8.28CDE
28/02/2020	7.30DEF	4.77GH	-	8.76BCDE
06/03/2020	6.28FG	3.02IJ	-	9.30BCD
13/03/2020	4.72GH	2.20IJK	-	10.03ABC
20/03/2020	3.64HI	1.41IJK	-	10.63ABC
27/03/2020	1.90JKL	0.77MNO	-	11.22AB
03/04/2020	0.99LMN	0.30NOP	-	12.17A
10/04/2020	0.63MNOP	0.04OP	-	12.68A
17/04/2020	0.44NOP	0.00P	-	12.65A
Mean	3.83B	2.13C	-	10.63A
Reduction%	99.96%	100%		

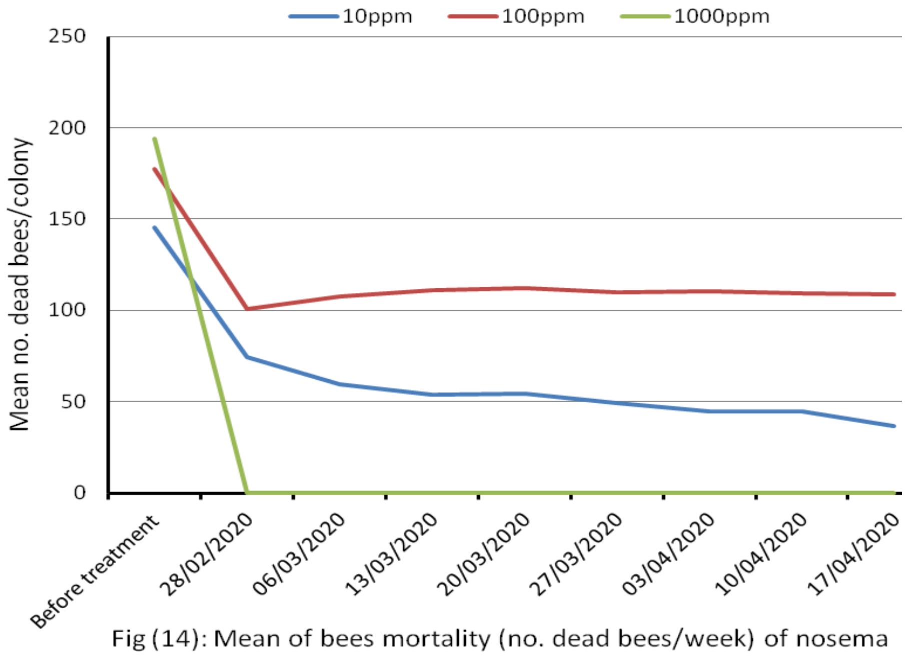

Fig (14): Mean of bees mortality (no. dead bees/week) of nosema infected colonies treated by sodium chlorite during season 2020.

Table(15): Mean of bees mortality (no. dead bees/week) of nosema infected colonies treated by sodium chlorite during season2021.

DATE	Sodium Chlorite ($NaClO_2$)			
	10ppm	**100ppm**	**1000ppm**	**Control (sugar syrup)**
Before treatment	155.32J	156.00J	185.20	184.0I
25/02/2021	87.32L	105.32KL	-	226.7H
03/03/2021	62.66M	110.66KL	-	268.0G
10/03/2021	56.66MN	117.32K	-	306.0F
17/03/2021	48.66MN	118.00K	-	341.3E
24/03/2021	45.32MN	114.00K	-	389.7D
31/03/2021	38.00N	111.32KL	-	431.3C
07/04/2021	36.00N	108.00KL	-	468.0B
14/04/2021	32.66N	103.32KL	-	522.7A
Mean	62.51C	115.99B	-	348.6A
Reduction%	92.59%	76.68%		

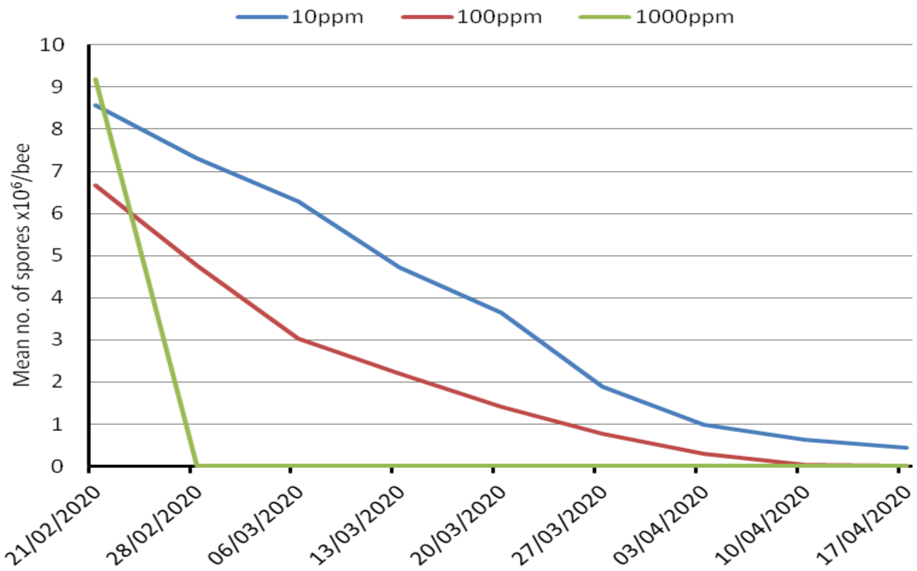

Fig (15): Mean of spores count (no. spores $x10^6$/bee) of nosema infected colonies treated by sodium chlorite during season 2020.

Table (16): Mean of spores count (no.sporesx106/bee) of nosema infected colonies treated by sodium chlorite during season 2021.

DATE	Sodium Chlorite(NaClO2)			
	10ppm	**100ppm**	**1000ppm**	**Control (sugar syrup)**
Before treatment	10.14BDCE	8.33EF	9.26	8.41EF
25/02/2021	9.28DE	7.97EF	-	9.22DE
03/03/2021	8.02EF	6.85FG	-	9.92CDE
10/03/2021	6.51FGH	5.67GHI	-	10.49BCDE
17/03/2021	5.43GHIJ	4.83HIG	-	11.05ABCD
24/03/2021	4.09IJK	3.84JK	-	9.82ABCD
31/03/2021	3.17KL	2.75KLM	-	12.26ABC
07/04/2021	1.93LMN	1.56MN	-	10.17AB
14/04/2021	0.91NO	0.00O	-	13.35A
Mean	5.50B	4.64C	-	10.52A
Reduction%	94.34%	100%		

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الملخص العربي

تقييم فعالية بعض المواد الكيميائية في علاج مرض النوزيما في نحل العسل تحت ظروف محافظة أسوان

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أجريت هذه الدراسة خلال العامين 2020–2021 لتقييم فعالية أربعة مواد كيميائية في علاج مرض النوزيما التي تصيب طوائف نحل العسل بمدينة كوم أمبو – بأسوان– مصر، حيث تمكنت الأربعة مواد من خفض الإصابة في الموسمين كالتالي: جزيئات الفضة النانوية و كانت أفضل النتائج عند 25 جزء في المليون حيث انخفض موت الشغالات بنسبة 99,21– 98,48%؛ أما تعداد الجراثيم انخفض بنسبة 100 – 100% في الموسمين على التوالي. أما كلوريت الصوديوم كانت أفضل نتائجه عند تركيز 10 جزء في المليون حيث انخفض موت الشغالات بنسبة 90,20 – 92,59% تعداد الجراثيم انخفض بنسبة 99,96 – 94,34% في الموسمين على التوالي. خفض موت الشغالات بنسبة 76,20 – 76,68% في الموسمين على التوالي ويلاحظ أن التركيز 10 جزء في المليون أفضل من حيث موت الشغالات حيث انخفضت بنسبة 90,20 – 92,59% في الموسمين على التوالي. أما بالنسبة لهيبوكلوريت الكالسيوم كانت أفضل نتائجه عند تركيز 100 جزء في المليون حيث انخفض موت الشغالات بنسبة 99.00–99.08%؛ أما تعداد الجراثيم انخفض بنسبة 100– 100% في الموسمين على التوالي. اليود المائي خفض موت الشغالات بنسبة 82,17–86,30%؛ و انخفض تعداد الجراثيم بنسبة 97,57–90,39%. على الرغم من فعالية الأربعة مركبات في علاج النوزيما إلا أنه قد نتج عنها بعض الأعراض السلبية في التركيزات الأعلى. فمثلا؛ عند تركيز 1000 جزء في المليون من كلوريت الصوديوم، اليود المائي تسبب في موت الطائفة. أما هيبوكلوريت الكالسيوم فقد تسبب في اختفاء الملكة و ظهور الشغالات الواضعة للبيض عند نفس التركيز. و بوجه عام أظهرت الدراسة أن الأربعة مركبات فعالة كعلاج لمرض النوزيما حيث تمكنت من خفض شدة الإصابة و تمكين الطائفة من استعادة نشاطها الطبيعي.