DIRECT INJECTION TECHNIQUE FOR BIO-PESTICIDE SPRAY

Sehsah, E.M.* and E.B.Belal**

ABSTRACT

Chemical pesticides have been used in agriculture and horticulture for several decades as a brute force method of achieving pest control. As a result, the persistence of some of these chemicals has left a long-lasting impact on the environment and non-target organisms. Pollution, pest resistance, and possible implications to cancer and diseases are a few such impacts. Modern pest control is shifting away from reliance on persistent chemical pesticides in favor of a more integrated approach to pest management. Applied biological pest control is an important component in an Integrated Pest Management (IPM) program. The main objective of the current research is reducing the organism damage due to these individual mechanisms in conventional agricultural spray application system by using the direct injection of bio-pesticide. The tequniche of biopesticied (Bacillus Thuringiensis . BT) was constructed and developed in Agriculture engineering dept. kafr El.sheikh University. The result indicate the mean values of B.T. spores for direct injection and Albuz B nozzle were 4×10^6, 6×10^7 and $8x10^7$ at operating pressure 250 kPa, 150 kPa and 100 kPa respectively. Further more, the values of B.T. at the same operating pressure for indirect sprayer were 1×10^5, 4×10^5 and $8x10^5$ at operating pressure 250 kPa, 150 kPa and 100 kPa respectively

Keywords: *Direct injection, Spray, Bio-pesticides*

INTRODUCTION

Over the past few years, the successful use of isolates of Bacillus thuringiensis (B.t.) to control many agricultural and vector insect pests, has stimulated world-wide interest in the design of research programs that aimed at finding even more potent fermentations.

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Unfortunately, this unhappy picture is most pronounced in developing countries and Egypt is no exception. This is associated with extensive use of chemical pesticides. Intensive research programs will have to be embarked upon to explore new avenues and develop non conventional methods of pest control and to advocate and strongly adhere to a sound program of pest management.

Currently, research is being undertaken on the use of the biological control agent Bacillus thuringiensis against agricultural pests at the National Research Centre, Cairo; other research institutions and Egyptian Universities. Microbial Pest Control Agents (MPCAs), notably products of various Bt subspecies, are increasingly used in pest management programmes against the larvae of several insect pests of major agricultural crops and forests, and several insect vectors of human diseases, and some nuisance pests. Bt products have been used worldwide, and their commercial production is about 1% of that of chemical pesticides. A number of reviews have recently been published on various aspects of Bt (McClintock et al., 1995; Kumar et al., 1996; Schnepf et al., 1998). Biological pesticides (i.e., bio pesticides) are receiving increased attention as benevolent alternatives to conventional chemical pesticides and as key components of integrated pest management (IPM) programs (Copping and Menn, 2000). Overuse of some chemical pesticides, resulting in pest resistance, and a reduction in the number of chemicals registered for use, and public concern for safety and environmental quality has accelerated interest in alternative pest management practices. Yet, few bio-pesticides are currently being used commercially as alternatives to chemical pesticides (Gan-Mor and Matthews, 2003), representing little more than 1% of the total world pesticide market (Menn and Hall, 1999). In contrast to chemical pesticides, bio pesticides are living systems (e.g., bacteria, fungi, viruses, predators, parasites), which introduces additional challenges with respect to formulation and delivery because the biological agents must remain viable during the application process to be effective. At present, there are few research-based guidelines on how bio-pesticides should be applied to optimize their performance in the field. Such guidelines are critical for increased acceptance and use of bio-pesticides by growers. Commercially, the implementation of bio -pesticides is most likely to come about with the development of products that can be applied using existing, conventional spray application equipment, as growers are unlikely to invest in new equipment or radically alter their practices (Bateman, 1999). In a conventional hydraulic spray system, the suspension is pumped from a tank reservoir, through pressure regulators and flow valves, to a nozzle where the suspension is forced under high pressure through an orifice to the atmosphere. A variety of hydrodynamic stresses are developed during flow through the spray system. In some cases, the stresses may be large enough to disrupt the structural membrane of an organism, causing permanent damage or death. Understanding the hydrodynamic stresses within a spray system is important to begin identifying the equipment characteristics and operating conditions that are least detrimental to the biological agents. The wide variety of spray equipment components commercially available makes it impossible to individually test each component for compatibility with each biological agent under varying operating conditions. Important flow field parameters from the numerical simulations can then be evaluated to determine whether the conditions within a particular equipment component are suitable to avoid hydrodynamic damage to the organisms. Because of its practical and theoretical appeal, the scalar quantity of energy dissipation rate has been used in several studies to characterize local hydrodynamic conditions resulting in cell (Ma et al., 2002) and organism damage (Fife et al., 2003a and Fife et al., 2003b). Application of bio-pesticides for use in crop protection continues to be an area of limited success. There have been reports of specialized release equipment being developed for specific organisms (Giles and Wunderlich, 1998). However, the specialized equipment has seen limited success in the field and remains in the hands of researchers and collaborators. At this point, no special apparatus has been developed and successfully marketed to meet the demands of the organisms during the application process (Nilsson and Gripwall, 1999; Penn, 2000). In terms of commercial acceptance, if specialized release equipment is a prerequisite for the use of a bio-pesticide, then the likelihood of the product to be widely used may be greatly reduced (Chapple and Bateman, 1997). Thus, the use of conventional agricultural spray application equipment for delivery may be necessary for widespread use of bio pesticides to occur. Macroscopic biological pest control organisms are several orders of magnitude larger than microbial agents, and in many cases, much more sensitive to damage in mechanical spraying systems. Thus, most releases of these organisms are in practice being done by hand. Some producers have developed their own techniques for dispersal, such as gas powered yard leaf blowers or a small remote controlled helicopter with a hopper attached for dispersal (Nielsen et al, 1998). Nilsson and Gripwall (1999) stated that the reason for the decreased viability in their study was probably mechanical stress from the pumps and nozzles. A conventional agricultural spray application system consists of three main equipment components. First, the spray tank where agitation takes place to keep the liquid suspension thoroughly mixed. Because of the nature of a bio-pesticide suspension, the possibility of the organisms falling to the bottom of the spray tank may make mixing an even more important consideration than with conventional pesticides. Typically, a pump withdraws part of the fluid from the tank and then injects it back into the tank through high-velocity jets from nozzles creating turbulent mixing in the tank. During operation, the liquid suspension will be reticulated through the pump many times. The second equipment component is the pump. Previous work has investigated the effect of mechanical pumping on organism damage. Based on this information, there appears to be a difference in the effect of mechanical pumping and type of biological pest control organism. Pumps with high mechanical shearing, pressure regulators, and control valves may damage the organisms by tearing them apart. Generally, a minimum pressure of at least 14.5 psi (100 kPa) is needed to overcome the surface tension of the liquid and provide sufficient velocity for the droplets to form. Droplet sizes and extent of coverage are extremely important factors for chemical pesticide application (Matthews, 1992); however, the relative importance of these factors for bio pesticides is less founded (Lello et al., 1996; Mason et al., 1998). At present, there are few research-based guidelines on how biological pest control agents should be applied to optimize their performance. Little information is known on how the different components of a spray application system affect the well being of biological pest control agents during the application process. One approach is to evaluate each component individually. Information on the effects of a hydraulic nozzle on organism damage is needed. The disruptive mechanisms that may be associated with the generation of high pressure and high velocities that occur during flow through a nozzle orifice include fluid shear, extensional flow, and a pressure differential. Organism damage due to these individual mechanisms is too difficult to distinguish during flow through the nozzle.

MATERIALS AND METHODS

This study was designed to reduce the microorganisms' damage by using the direct injection system. The direct injection system presented as shown in figures 1 &2 and used to investigate the direct injection of the B.T (*Bacillus Thuringiensis*) under laboratory operating conditions for temperature 20° C and relative humidity 70 %. The basic principle of direct injection on a crop sprayer is that pesticide and water is kept in separate containers. When the sprayer is activated, a metered flow of pesticide is injected into the water stream, sometimes via a mixing chamber, at a point situated between the main water tank and the nozzles. Often a number of pesticide containers and pumps are fitted to allow the farmer to apply more than one product. The components of the direct injection system consisted of the programmable flow controller, pressure gauge manometers, water tank 50 L, bio-pesticides tank 5 l, water pump 0.6 kW, compressor 2.8 kW, valves and regular pressures as shown in figure 2. The operating pressure was calculated that a pressure of 30 kPa above the water pressure. It's was needed to allow a flow rate of 4.7 l/min of the pesticide into the mixing chamber. This calculation was based on the pesticide having the same density as water, and does not allow for the different viscosities. The air source will have to be a steady supply, so that the air regulator is not affected. To achieve this there will have to be a compressor and a suitably sized air receiver to smooth out any pulsations. The compressor should be sized so that it can easily cope with the maximum flow rate of 4.7 l/min, and the maximum pressure of the compressor should be above the maximum air pressure requirement for the system. The compressor was driven by a 2.8 kW electric motor. The size of two water tanks is 50*50*40 cm with total capacity 100 L and the volume of mixed chamber is 0.4 L (5 cm inside diameter and 21 cm length). The chamber was made from Plexiglas that can be able to see the mixing solved material. The direct injection system included the one way valves at main point injection for liquid water and two way valves for bio-pesticides. As well as, the flow liquid controller set up between the regular valve and the mixing chamber. The different type of nozzles Abutz B, TT DG 8004 and LU 130-03 were selected to study their effect on BT for direct and indirect injection system.

Bacillus thuringiensis

Bacillus Thuringiensis (B.t.) is the most promising agent for control of insects of economic and medical importance. BT is a facultative anaerobic, motile, gram-positive, spore-forming bacterium. The formation of parasporal crystals adjacent to the endospore during sporulation stages III to IV distinguishes BT from other Bacillus species. BT, like other Bacillus species, has been classified on the basis of its cellular, cultural, biochemical and genetic characteristics (Hansen et al., 1999). The bio-alternatives include specific narrow spectrum chemicals like pheromones and entomopathogens i.e. viruses, bacteria and fungi. These bio-rational insecticides are unique because they have a narrow spectrum of action and literally no toxicity to non-target vertebrates and invertebrates. Spore forming bacilli belonging to Bacillus thuringiensis (B.t.) species are a major group which produce pro-toxins with molecular weight ranging between 110 to 200 KDa, during sporulation. These proteins are deposited either as parasporal inclusions in these bacilli or in some cases found on the surface of spores. Due to the applications of molecular biology, the crystal toxins of B.T. became better characterized and currently effective combinations of B.T. strains for diverse pests are evolving.

Biological treatment of green rice straw

Bacillus thuringiensis was grown and prepared in the laboratory of microbiology, faculty of agriculture, Kafr El Sheikh University. With shake flask cultures, *Bacillus thuringiensis* was grown on nutrient broth medium (contents per liter, 5 gm glucose, 5 gm yeast extract, 5 gm peptone and 5 gm sodium chloride) at 30 °C and 150 rpm for 3 days. Cells number of the bacterial strain was determined by plating appropriate dilutions of liquid medium onto nutrient agar before and after sprayed. Dissolve in 800 ml distilled water and the pH was adjusted to 7.5 and make up to 1000 ml.

The Image J software Version 1.52 used to count the BT spores cells by capturing and detecting the BT cells with microscope PC camera. As well as, the standard methods of cell counted used and compared with the cell counted by the imaging processing program (Image J software Version 1.52) figure 3. The result indicated that it could be able to use the Image J program to count the BT and it will be easy method as shown in figure 4.

Procedure and measurements

The direct injection system was tested and adjusted before applied the BT by using the BSF fluorescent dye. BSF fluorescent dye was used at a concentration of 0.1 %. The sample from the sprayer's tank was collected for calibration of the measurement. The tracer concentration in the washing solution was determined using the Fluorescence Spectrometer. The two different nozzles were fixed at end of the direct injection system at boom sprayer. The two cylinders were fixed under each two nozzles to collect the samples. The samples were collected after the treatments to measure the concentration. As well as, the flow rate from the each nozzle was measured at every treatment. The regular pressure at air compressor used to control of the liquid flow rate in compressed air Bio-pesticides tank. After calibration of the direct injection system, the BT with concentration of 5 $x10^8$ cell / ml was feed into the compressed air (Bio-pesticides) tank and injected into the maxing chamber at injected point. As well as, all experimental tested and adjusted at three different operating pressure 100 kPa, 150 kPa and 250 kPa. The compressed air in the bio-pesticides tank was above 30 kPa of water pressure. The conventional sprayer was setup by remove the maxing chamber and Bio-pesticide tank from the direct injection spray system. The all above same treatment conditions in the direct injection were tested in the conventional sprayer (indirect injection system). The conventional sprayer (indirect sprayer system) was used to compare with the direct injection system to indicate the effect of the direct injection sprayer on the BT.

RESULTS AND DISCUSSIONS

The experimental randomization completely design was designed with three replication to investigate the effect of two techniques liquid injection and their conditions on B.T under laboratory conditions.

Operating pressure and their effect on the B.T spores

Figure 5 indicates the effect of operating pressure on the number of B.T spores/ml for direct injection spray system. The high values of operating pressure gave low values of number of B.T spores/ml i.e. reduction of the organisms. It is clear that, the damage of B.T. spores tend to increase by increasing the operating pressure because the operating pressure shearing or affected on the B.T. cells through the orifices of nozzles. The minimum value of B.T spores for direct injection due to increase the operating pressure was found of Albuz B nozzle. The mean values of B.T. spores for direct injection and Albuz B nozzle were 4×10^6, 6×10^7 and $8x10^7$ at operating pressure 250 kPa, 150 kPa and 100 kPa respectively. Further more, the values of B.T. at the same operating pressure for indirect sprayer were 1×10^5, 4×10^5 and $8x10^5$ at operating pressure 250 kPa, 150 kPa and 100 kPa respectively as shown in figures 5 and 6. Analysis of variance of the operating pressure was a highly significant effect on the damage of B.T. spores. The high operating pressure increased the damage of B.T spores.

Effect of nozzles on B.T. spores

The Albuz B nozzle gave the high values of B.T. spores compared to TT DG 8004 and LU 130-03 nozzles at low operating pressure 100 kPa for direct injection. Also, figures 5 and 6 illustrate the effect of Albuz B, LU 130-03 and TT DG 8004 nozzles at 100 kPa, 150 kPa and 250 kPa on B.T. spores for direct and indirect injection system. It is clear that, the large orifice and low pressure for nozzles gave low damage of B.T compared to small orifice and high pressure for both injection systems. The TT Dg 8004 nozzles gave the highest damage values for bio-pesticides (B.T.) compared with the Albuz B and LU 130-3 nozzles at all operating condition for the indirect injection system. The B.T spores value for TT Dg 8004 nozzle was 1×10^6 at operating pressure 250 kPa under indirect injection system treatment as shown in table 1.

Effect of direct and indirect injection spray system on the B.T spores

Direct injection systems for pesticide application keep the pesticide and carrier (water) separate while metering and mixing them on demand within the pipeline before entering the nozzle. The obtained data presented in Table 1 indicate the effect of injection systems types of B.T. cells on reduction values of organisms (B.T spores /ml). The direct injection spray system gave the high values of B.T spores/ml comparing with the indirect injection spray system at the all operating pressure and for all nozzles. The mean values of B.T spores for direct injection system at 100 kPa were 8×10^7, 7×10^7 and 4×10^7 for nozzles Albuz B, LU 130-03 and TT DG 8004 respectively. Similarly, the B.T spores values of indirect injection system at the same conditions100 kPa and three nozzles were 4×10^6, 4×10^6 and 8×10^5 for nozzles Albuz B, LU 130-03 and TT DG 8004 respectively. The force will increase correspond the shear stress on the fluidized B.T cells for indirect injection spray system.

Summary and Conclusions

In this laboratory experiment it could be shown that a low pressure atomization (100 kPa) of living organisms mixed in water is possible. Particularly the results of the direct injection spray system are very promising. The reduction damage of B.T cells (bio-pesticides) which are already below the standard field application can even be improved at the expense of decrease the operating pressure. With reference to a comparable direct injection gave reasonable values of reduction damage of B.T cells compared with the indirect injection spray system at all operating pressure. The round orifice of the Albuz B nozzle seems to be easy to the B.T cells. With the hydraulic spray of course there is a possibility to further improvement by increasing nozzles size and correspondingly changing the injection system to direct injection spray system. The type of nozzles and operating pressure was highly significant effect on the damage *B T bio-pesticides*. As well as, the direct injection spray system can even be improved the main carrier tank is loaded with clean water, not mixed pesticides materials. Concentrated pesticides is accurately injected and mixed just prior to being sprayed out of the boom. As well as reducing the operator exposure to chemical concentrates. No pre-mixing of pesticides materials in the main carrier tank. No leftover mixed product at the end of the field to dispose of unused concentrated chemical remains safe and secure in a dedicated holding tank. The operator can quickly change from one pesticides materials product to another without cleaning and rinsing the main carrier tank. Chemical application rates can be adjusted by varying the concentration of pesticides materials injected into the carrier. The injection process needs to be examined to discern to what extent injection quality is affected by various pressures, viscosities and valve adjustments. Also, a rinsing system which removes pesticides from the inner surfaces of the pipes must be developed.

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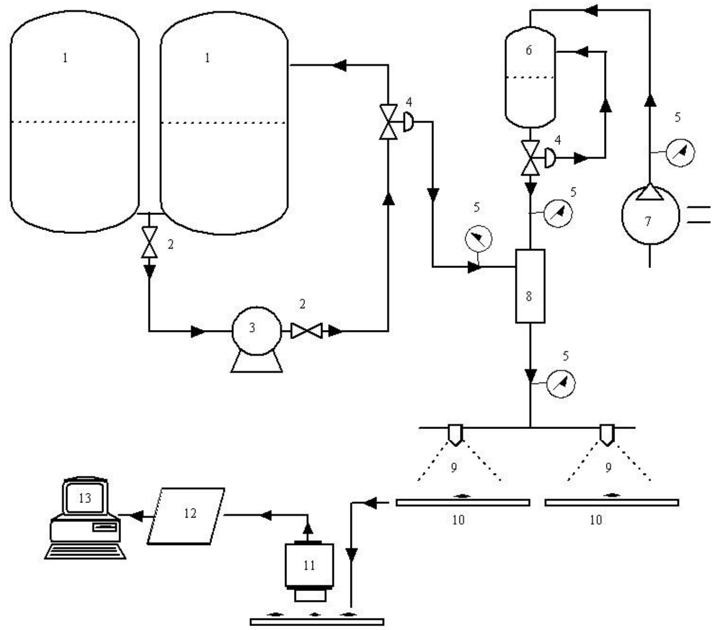

1-Water tank | **2- Gate valve**
3- Water pump | **4-Two way valve**
5- Pressure gauge | **6- Bio-pesticides tank**
7- Air Compressor | **8- Mix-chamber**
9- Nozzles | **10- Collector for Organisms**
11- Microscope PC camera | **12- Image J program Vs.1.46a**
13- PC

Fig. (1): Presented the diagram of the direct injection system for bio-pesticide (B.T)

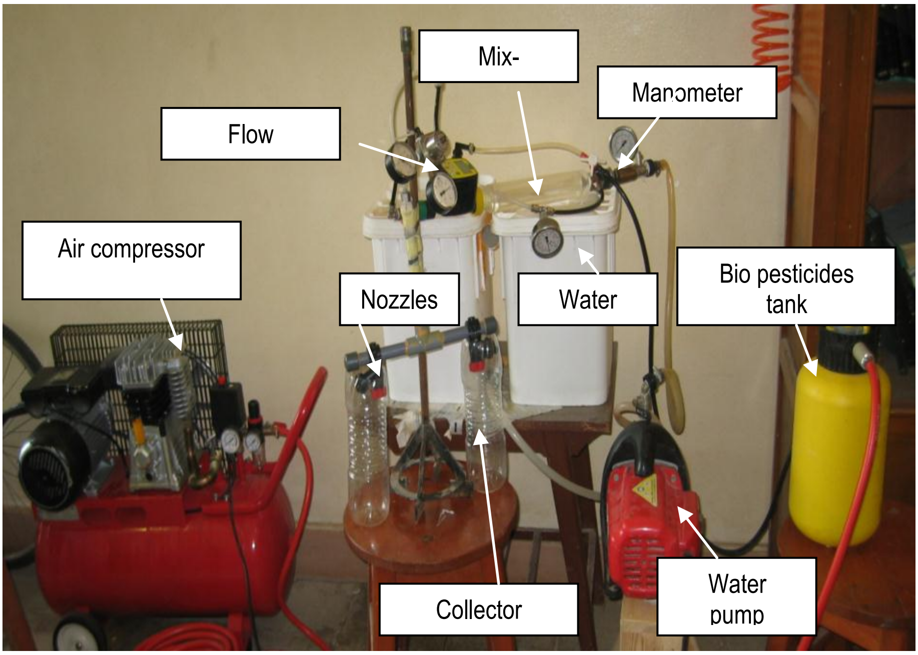

Fig. (2): Presented the direct injection system for bio-pesticide (B.T) in the laboratory.

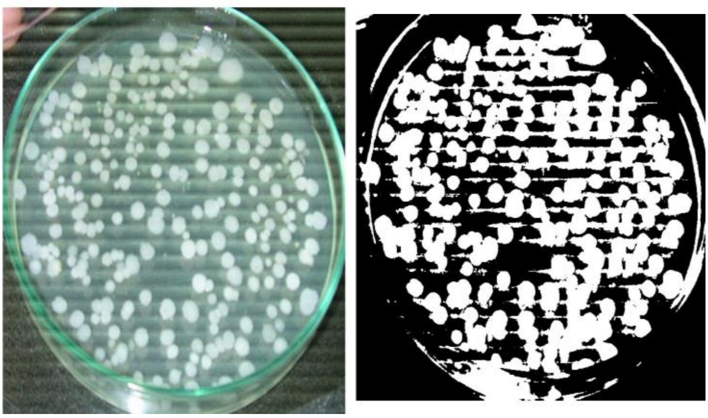

Fig. 4: Indicate the B.T Spores photo after 24 h sprayed and converted to imaging processing programme Image J V.1.52

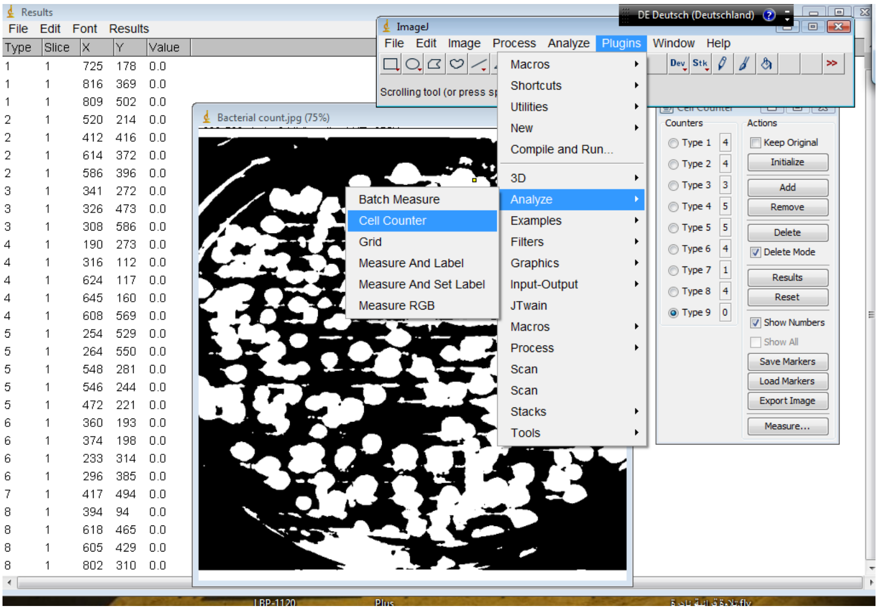

Fig. 3: Indicate the imaging processing programme Image J V.1.52 to count the B.T spores

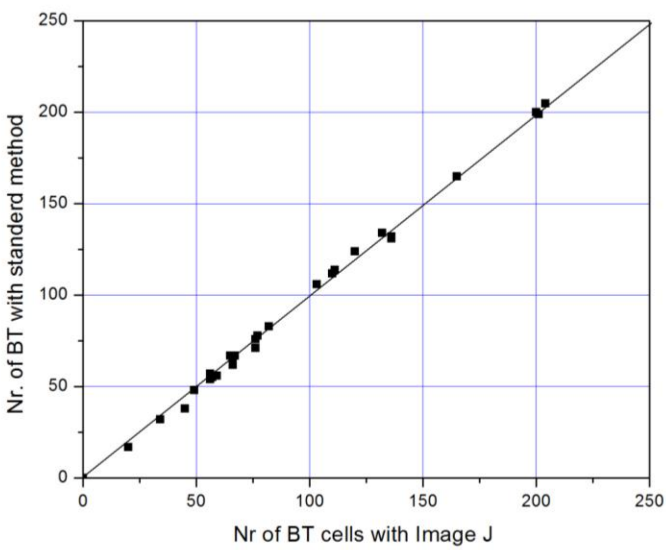

Fig. 4: Display the compared tow different methods of counting the B.T

Table 1: Presented the effect of injection system spray at three operating pressure for on B.T spores.

Operating pressure, kPa	Direct injection system			Indirect injection system		
	Albuz B	LU 130-3	DG 8004	Albuz B	LU 130-3	DG 8004
100	$8x10^7$	$7x10^7$	$4x10^7$	$4x10^6$	$4x10^6$	$8x10^5$
150	$6x10^7$	$4x10^7$	$2x10^7$	$3x10^6$	$2x10^6$	$4x10^5$
250	$4x10^6$	$2x10^7$	$1x10^7$	$4x10^5$	$1x10^6$	$1x10^5$

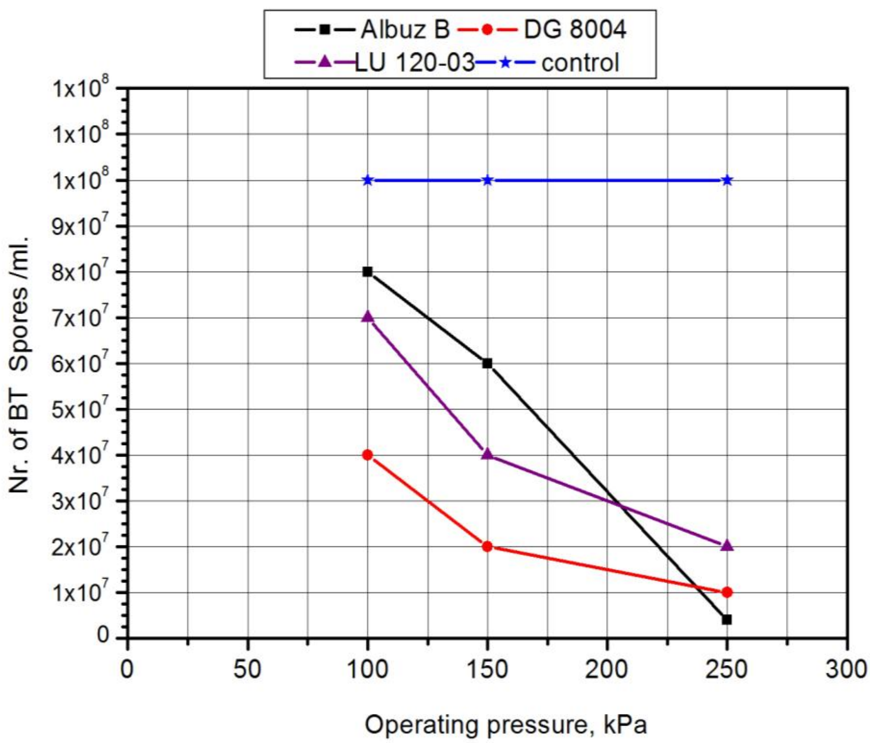

Fig. 5: Presented the effect of operating pressure and type of nozzles for direct injection spray system on B.T spores/ml

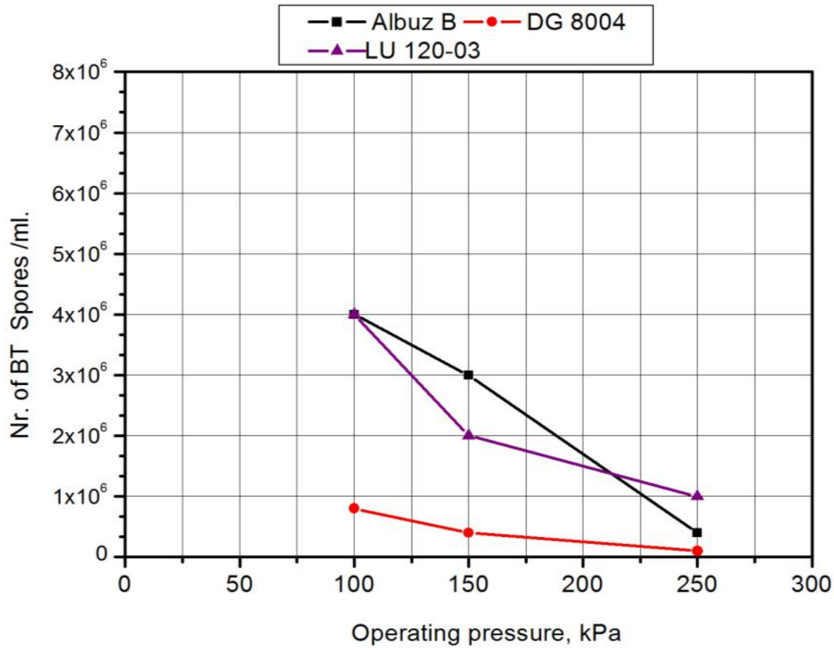

Fig. 6: Presented the effect of operating pressure and type of nozzles for direct injection spray system on B.T spores/ml

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الملخص العربى

تقنية الحقن المباشر لرش المواد البيولوجية

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مما لا شك فيه أن أستخدام المبيدات الكيمائية لها تأثيرها الضار على الأنسان و البيئة . و لقد تزايدت فى الآونة الآخيرة أستخدام المكافحة الحيوية فى برامج المكافحة المختلفة فى مصر و العالم و منها أستخدام البكتريا .B.t . و تهدف هذه الدراسة إلى البحث في إمكانية الحقن المباشر للمواد البيولوجية المستخدمة في المكافحة الحيوية مثل البكتريا BT وذلك بغرض الحد من الفواقد التي تنشأ عند استخدام آلات المكافحة التقليدية (الرشاشات ذات الحقن الغيرمباشر) مثل الأضرار الميكانيكية و الفزيائية أثناء سريان المواد البيولوجية داحل أجزاء آلة الرش التقليدية. حيث أجريت هذه الدراسة في معمل قسم الهندسة الزراعية كلية الزراعة بكفر الشيخ وذلك بتطوير وحده للحقن المباشر للمبيدات التي تعتمد على إضافة سائل الرش البيولوجي و خلطه في غرفه تم تركيبها قبيل الفواني. و تتلخص الفكرة الأساسية في نظام الحقن المباشر للسوائل و منها السوائل المستخدمة في المقاومة البيولوجية (BT) ، بوضع المبيد في خزان منفصل صغير للمبيد هو عبوة المبيد نفسة و الخزان الرئيسي للرشاشه يستخدم فقط فيه الماء و يتم التحكم في عملية الخلط عن طريق إضافة المبيد (السائل البيولوجى البكتيرى BT) التي تم إعدادها في معمل قسم الميكروبيولوجي بكلية الزراعة جامعة كفرالشيخ إلى تيار المياه في غرفه للخلط مصنعة من Plexiglas الشفاف حيث تم تركيبها و وضعها قبيل الفواني وكان طولها ٢١ سم و قطرها الداخلى ٥ سم.

و تتكون وحدة الحقن المباشر من خزان رئيسي للمياه فقط مقسم إلى جزئين بسعة ٥٠ لترللواحد و طلمبه طارده مركزية قدرة ٠.٦ ك.وات تعمل الطلمبة بسحب الماء من الخزان وطرده إلى غرفة الخلط حيث تم تركيب صمام ذو إتجاه واحد في نهاية خرطوم الطرد عند نقطة الحقن للطلمبه. و تتكون الوحده أيضاَ من خزان أخر للمبيد بسعة ٥ لتر حيث يتصل الخزان من أعلى بخرطوم للهواء للضغط على سطح المبيد حيث تم و صله بضاغط هواء Commpressor of air ذو قدرة 2.8 kW و الذى يحتوي على منظم لتحديد ضغط الهواء المطلوب لإزاحة سائل الرش الموجود في خزان المبيد و كذلك تم تركيب صمام ذو إتجاهين في نهاية خرطوم الطرد الخاص بالمبيد و عند منطقة الخلط فى غرفة الخلط.

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كما أشتمل نظام الحقن المباشر على أجهزة لقياس ضغط Pressure gauges لكل من سائل الرش المراد خلطه و المياه المستعمله قبل و بعد عملية الخلط و كذلك صمامات للتحكم. كما تم تركيب منظم programmable flow controller لسائل الرش للتحكم في معدل الرش الخارج من غرفة الخلط إلى الفواني و كذلك تم تركيب منظم للضغط قبل الفوانى. كما استخدم ثلاثة أنواع من الفواني هى Abutz B, TT DG 8004, LU 130-03 لإختبارها ودراسة تأثيرها على سائل الرش (BT).

- **<u>الإختبارات الأولية وتقييم وحدة الحقن المباشر</u>**

قبيل البدء في دراسة استخدام نظام الخلط المباشر لحقن سائل الرش أستخدمت الصبغة BSF كبديل للمبيد تحديد إمكانية الخلط وذلك بتقييم نسبة الخلط الناتجه. حيث أضيف BSF بتركيز ٠.١ % وتم حقنه خلال وحدة الحقن وتجميع سائل الرش عند فرق ضغط بين سائل الرش و ضغط حقن الماء بزيادة مقدارها ٣٠ ك بسكال لضغط المبيد عن ضغط الماء. كما أستخدم جهاز Spectrophotometer لقياس تركيز سائل الرش الناتج عند كل ضغط من ضغوط التشغيل موضع الدراسة وهما ١٠٠، ١٥٠، ٢٥٠ ك بسكال.حيث وجد أن تركيز BSF المستخدمة قل بنسبة ٤% عن التركيز المطلوب.

<u>تقدير عدد (BT)</u>

لتقدير عدد البكتريا (BT spores/ml) الناتجه بعد مضى ٢٤ ساعة من عملية الرش حيث أن هذه الفترة تتحول فيها خلايا الBT الى مادة سامة للحشرات المطلوب مكافحتها و هو ما يعرف بالBT spores ، استخدمت الطريقة التقليدية في العد ولقد تم مقارنة ذلك بأستخدام برنامج معالجة الصورImaging لعد خلايا(BT) حيث أخذت مجموعة من الصور بعد عملية الرش و معالجتها ببرنامج المعالجه للصور وتحويل الصوره المأخوذه إلى Binary لتقدير عدد BT spores عند كل معالجه. أظهرت النتائج الأولية أنه يمكن استخدام برنامج معالجة الصورفي تقدير و عد BT Spores حيث كانت الفروق غير معنوية وذات معامل أرتباط عالى.

- **<u>إختبار وحدة الحقن ودراسة تأثير الحقن المباشر والغير مباشر على (BT) المستخدمة فى المكافحة الحيوية</u>**

أجريت هذه الدراسة تحت ثلاثة ضغوط مختلفة هما ١٠٠، ١٥٠، ٢٥٠ بسكال وعند معدل تصرف من وحدة الحقن بمعدل٤.٧ لتر/دقيقه حيث تم حقن الـ(BT) و التحكم في ضغوط الحقن لكل من سائل الرش (BT) و الماء بزيادة قدرها ٣٠ كيلو بسكال لضغط الهواء المستخدم فى خزان المبيد ، كما تم ضبط وحدة التحكم في تصرف السائل المتجه إلى الفوانى و تجميع سائل الرش الناتج (BT) لتقدير عدد BT و التى تم إضافتها بتركيز^{٨}١٠×٥ B.T cells و لأجراء إختبار تأثير طريقة الحقن الغير مباشر (الرشاشه الهيدرولكية التقليديه) ، تم فصل خزان المبيد وغرفة الخلط عن النظام وتشغيله على انه نظام حقن غير مباشر اي رشاشه هيدروليكية تقليدية. و لقد أجريت نفس المعاملات السابقه التى تم دراستها في الحقن

المباشر على الحقن الغير مباشر كما تم تجميع سائل الرش الناتج من الفواني الثلاثة موضع الدراسة لتقدير عدد الـ BT spores / ملى لترالناتجه من الرش.

أهم النتائج المتحصل عليها

في دراسة تأثير نوع نظام الحقن المستخدم في حقن المواد البيولوجيه (BT) أتضح ان هناك تأثير عالي المعنويه حيث أعطى الحقن المباشر للبكتيريا (BT) أعلى عدد من BT-Spores / ملى لتر و التى هى دالة لجودة عملية الرش مقارنة بالحقن الغير مباشر أي استخدام الرشاشات الهيدروليكية التقليدية. حيث وصل عدد خلايا BT-Spores / ملى لتر إلى $٨×١٠^{٧}$ للحقن المباشر مقارنة $١٠^{٦}$× ٤ فى الحقن الغير مباشر عند ضغط تشغيل ١٠٠ ك.بسكال.

كما أتضح أيضاَ من الدراسة أنه بزيادة الضغط يقل عدد الـ BT spores / ملى لترالناتجه حيث وصل أقل عدد $٤×١٠^{٦}$ للحقن المباشر عند ضغط ٢٥٠ ك. بسكال ، $١×١٠^{٥}$ للحقن الغير مباشر .

و اتضح أيضاَ من الدراسة أن لنوع الفونى تأثير على عدد الـ BT spores / ملى لترالناتجه حيث أنه بزيادة قطرفتحة الفواني فإن ذلك يساعد على الحد من التلف فى مادة BT الناتجه أو عدد خلايا البكتيريا الناتجه التي تعتبر دالة لكفاءة عملية الرش في المكافحة البيولوجية حيث بلغت قيم BT-Spores / ملى لتر $٨×١٠^{٧}$ ، $٧×١٠^{٧}$ و $٤×١٠^{٧}$ عند أقل ضغط للفوانى LU 130-03, Abutz B, و TT DG 8004 على الترتيب. ويمكن من خلال الحقى المباشر للمبيد فى صورة المختلفة البولوجية منها و الكيميائية الحد من تلوث البيئة بتقليل عمليات غسيل آلات الرش بعد كل عملية و بخاصة عند تفيير نوع المبيد كما أن فى الحقى المباشر يمكن تفيير نوع المبيد بسهولة و الحد من الفواقد التى تنشأ عن الجزء المتبقى من المبيد داخل الخزان و كذلك أمكانية خلط المبيد بسهولة و توفير الطاقة المتمثلة فى عملية التقليب داخل الخزان.