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EVALUATION OF CERTAIN SAMPLING METHODS FOR ADULTS OF *Bemisia* SPP. (Homoptera: Aleyrodidae) AND SOYBEAN STEM FLY, *Melanagromyza cunctans* MEIGEN (Diptera: Agromyzidae) ON SOYBEAN PLANTATIONS AT MANSOURA DISTRICT.

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ABSTRACT

Sampling of *Bemisia* spp. and *M. cunctans* was carried out at Mansoura district, Dakahlia Governorate during 2008 and 2009 seasons. We examined several types of yellow sticky cards (vertically oriented cylinder, vertically oriented two-sided traps and vertically oriented, one-sided traps), three colored of water pan traps (yellow, blue and red), the plastic cup trap (CC trap), sweep-net that indirectly measured adult abundance based on activity and visual examination (leave-turn) and black pan methods as two direct count sampling methods based on the census of adults on or from the plant.

The count of *Bemisia* spp. was highly significantly effect by sampling methods. Among the sampling methods, cylinder sticky traps was caught the most adults of whitefly during the two seasons. Furthermore, there were no significant differences between one sided sticky traps, CC traps, black pan, vacuum sampler, whole-plant, and leaf-turn method. In addition, there were no significant differences between cylinder sticky and two-sided traps. The black pan had the lowest associated relative variation (RV) value in both seasons. Whereas, the whole-plant had the highest calculated RV value in the first season. While in the second season, the cylinder sticky traps had the highest calculated RV value. The black pan method had the highest calculated RNP value (most efficient) compared with all other sampling methods. Whereas, the vacuum sampler had lowest associated RNV value in both seasons.

The count of *M. cunctans* was highly significantly effect by sampling methods. Among the sampling methods, cylinder sticky traps caught the most adult of *M. cunctans* during the two seasons. Furthermore, there were no significant differences between yellow water pan traps, visual examination, and sweep-net. Moreover, there was no significant differences between one sided sticky traps, whole-plant, blue water pan traps, red water pan traps and two-sided sticky traps. The cylinder sticky traps had the lowest associated RV value in 2008 season. Whereas, in 2009 season, the two-sided sticky traps had lowest associated RV value.

Keywords: *Bemisia* spp., *Melanagromyza cunctans***,** sampling methods**,** soybean

INTRODUCTION

Soybean, *Glycine max* (L.) is one of the most important food legume all over the world and plays a good role in various industries and nutritional

aspects for people and animal. Seeds of soybean have high nutritional value and their protein contain many essential amino acids (Gamieh and El-Basuony, 2001). Seeds have good nutritional quality and consider one of richest sources of oil (18-22 %). The area under cultivation in Egypt steadily expanded since1970 about 160,000 feddans (Hammed, 1977; Zarrif, 1989; and Mesbah and El-Galaly, 1999)

Whiteflies, *Bemisia* spp. complex and *Melanagromyza cunctans* Meigen are serious pests of soybean plantations. Whiteflies feeding extract important nutrients, caused defoliation and poor plant yield. This pest also causes several plant physiological disorders, such as tomato irregular ripening and transmitting serious virus diseases. The soybean stem fly, *M. cunctans* causes significant losses in soybean yield, quality and germination potential (Abdel-Salam *et al.,* 2005). El-Basiony *et al.* (1996) also reported that *M. cunctans* is a serious pest, causing 100 % infestation on soybean plantations; as a result seed yield is reduced causing seedling to die, while growth and yield in mature plant are reduced. It has been reported that 22 plant species from 6 families are attacked by *M. cunctans* in Northern Sinai.

Control measures, insecticides or biological control can only be applied in the most efficient way if sampling techniques give reasonably precise estimates of pest densities and occurrence, even at low densities. Accurate information on pest occurrence and densities can increase the efficiency of pesticides by properly timing their application. In this way, prophylactic spraying can be avoided, which is important as many agromyzid pests have been shown to develop insecticide resistance. By minimizing the application of a pesticide, its effective life can be extended (Scheirs *et al.,* 1997). Therefore, the objective of the current study was to establish the reliability of trapping data. In this study, we tested the reliability of different trap types for the assessment the abundance of *Bemisia* spp. complex and *M. cunctans.*

MATERIALS AND METHODS

Samplings of *Bemisia* spp. complex and *M. cunctans* were carried out at Mansoura district, Dakahlia Governorate during two consecutive seasons (2008 and 2009) during the soybean growing season. We examined several types of yellow sticky cards, three colored of water pan traps, Cc traps, and sweep-net that indirectly measured adults abundance based on activity and visual examination (leave-turn method) and black pan methods as two direct-count sampling methods based on the census of adults on or from the plant.

I. Sampling techniques:

Yellow sticky traps:

Three basic types of yellow sticky traps were evaluated during the 2008-2009 seasons; vertically oriented cylinder, vertically oriented two-sided and vertically oriented one-sided traps. The height and placement of the traps relative to the field were varied over the study. The cylinder traps were made by wrapping a sticky trap (22 by 10 cm.) around a plastic pipe that was then placed on a wooden stake. The two-sided trap measured (11 by 10 cm) and was positioned vertically on a wooden stake. The one-sided traps measured

(11 by 10 cm) and were oriented horizontally with the sticky surface facing skyward. The one and two-sided traps were stapled to plastic pot stakes and then attached to wooden stakes in the field. The stakes were placed within the row and plants were cleared as necessary to ensure that leaves did not become entangled on the sticky surface. The traps height was adjusted as needed throughout the season.

Black pan:

The black pan method is a modification of technique first described by Butler and Wilson (1986). It consists of tapping the top of soybean plant three times over black cake pan (22.9 by33 by 5.1 cm deep) coated with a thin layer of vegetable oil. In total, ten plants were tapped as the sampler walked down the row, and the trapped whiteflies were then counted. A grid etched into the bottom of the pan aided counted when densities were high.

Visual examination (leaf turn method):

The visual search method involved examination of the terminal and all structures beginning in the terminal and working down through the plant. All of *Bemisia* spp. complex and *M. cunctans* on leaves and stems also were recorded on 10 plants selected randomly during the examination. Adult of the two insects were found underside leaf which counted by carefully rotating the petiole or the tip of the leaf blade (Naranjo and Flint, 1995).

Whole plant:

The sample unit consisted of two plants chosen randomly from interior rows of a soybean plot. A white cylindrical bag of nylon (1 m diameter and 1.5 m long) with a drawstring at each end was lowered over the plant and folded flat on the ground so that it surrounded the base of the plant. The lower drawstring was tightened around the base of plant and the plant was cut at ground level and the bag containing the plant and insects was taken to laboratory. The bag and plants were frozen to kill the insects and then plants and bag were examined and counted all the insects (Byerly *et al.,* 1978).

Sweep-net:

Hundred double strokes were taken weekly from plants. Each collected sample was put into plastic bags and transferred to the laboratory. Specimens were anaesthetized by diethyl ether and examined. Numbers of the insect pests were counted.

The plastic cup trap (CC trap):

This trap was designed to capture *Bemisia* spp. adults for survey, monitoring, and sampling in the field. The trap design was based on whitefly adult behavioral attraction to yellow color, flight orientation to sky light when leaving host plant and walking to shade when landing on a new host for feeding and oviposition of eggs. The CC trap consisted of two components. The trap top is an 11.2 cm high, 350 ml crystal clear plastic drinking cup. The open cup end fits into a yellow plastic bas with a cylinder shape outside and hollow cone inside surface. The trap base is 7.9 cm outside and 7.1 cm inside; the top opening of the trap base has a 5.2 cm outside diameter and 4.8 cm inside diameter. The additional component is a circular clear plastic deflector plate with a diameter of 6 cm was mounted over the top tap base opening and was supported by four 3.7 cm long plastic legs. The gap between the trap base top opening and the plat is 1.5 cm. The hollow cone

trap base opening allows insect entrance and the deflector plate prevents trapped adult from escaping (Chi and Henneberry, 1998).

Colored water traps:

As attraction traps, we tested three different colored water traps: yellow, blue and red (Scheirs *et al*., 1997). All traps were filled with water. A few drops of a detergent were added to lower surface tension. The traps were emptied at weekly intervals throw the grown season. The numbers of *Bemisia* spp. complex and *M. cunctans* species in the different traps were counted.

Vacuum sampler:

A modified vacuum sampler procedure is used to collect insects. The procedure consists of moving the opening suction tube above the plants beginning at the top and ending at the bottom of the plant. Collection vials were transported to the laboratory and frozen and the number of adults was counted.

II. Statistical analysis:

Mean values for counts of *Bemisia* spp. complex and *M. cunctans* for all sample methods were determined using analysis of variance (ANOVA) (Costat, 2004). The level of precision per unit of cost was compared among the sampling methods (Buntin, 1994). Relative variation (RV) was used to measure the precision of the sampling method. RV was calculated as the percentage of mean standard error relative to the mean:

$$RV = (SEM / m) * 100$$

Where SEM is the standard error of the sample mean and m is the sample mean. Therefore, a smaller RV indicated greater precision. Southwood (1978) reported that RV ≤ 25 was suitable for extensive sampling programs. Relative net precision (RNP) was calculated and used to measure the efficiency of the sampling method (Buntin, 1994). RNP is measure that equally considers the precision of the sampling method and its cost typically expressed in labor time. It was calculated as follows:

$$RNP = [1 / (RVm)\ (C)] * 100$$

Where RVm is the mean relative variation and C is the cost to process one sample. Cost values were determined by averaging the time required to collect by averaging the time required to collect and count.

RESULTS AND DISCUSSION

***Bemisia* spp.**

As shown in Table (1), the count of *Bemisia* spp. was highly significantly effect by sampling methods. Figure (1) shows the weekly trap captures of *Bemisia* spp. as influenced by sampling methods. All sampling methods indicated similar population trends throughout the two seasons. Statistical analysis showed that the two-sided sticky traps caught significantly more adult of whiteflies. For all methods, adult populations were low early and increase later throw the 2008 and 2009 seasons. The results could be supported by the results of Palumbo *et al.* (1995) who pointed out the sticky traps consistently estimated the greatest number of *Bemisia* spp. adults.

There were significant differences between traps during 2008 and 2009 as shown in Figure (2). Among the sampling methods, cylinder sticky

traps was caught the most adults of whitefly during the two seasons Furthermore, there were no significant differences between one sided sticky traps, CC traps, black pan, vacuum sampler, whole-plant, and leaf-turn method. In addition, there were no significant differences between cylinder sticky and two-sided traps.

Table (1): One way analysis of variance (ANOVA) for the impact of sampling methods on the numbers of *Bemisia* spp. during 2008 and 2009 seasons at Mansoura district.

Factor	Sum of squares	Degrees of freedom	Mean square	F. Test	P
2008					
Method	104659.33	7	14951.33	19.451	0000***
Error	67640	88	768.636		
2009					
Method	51368.57	7	7338.36	10.27	0000***
Error	62851.41	88	714.22		

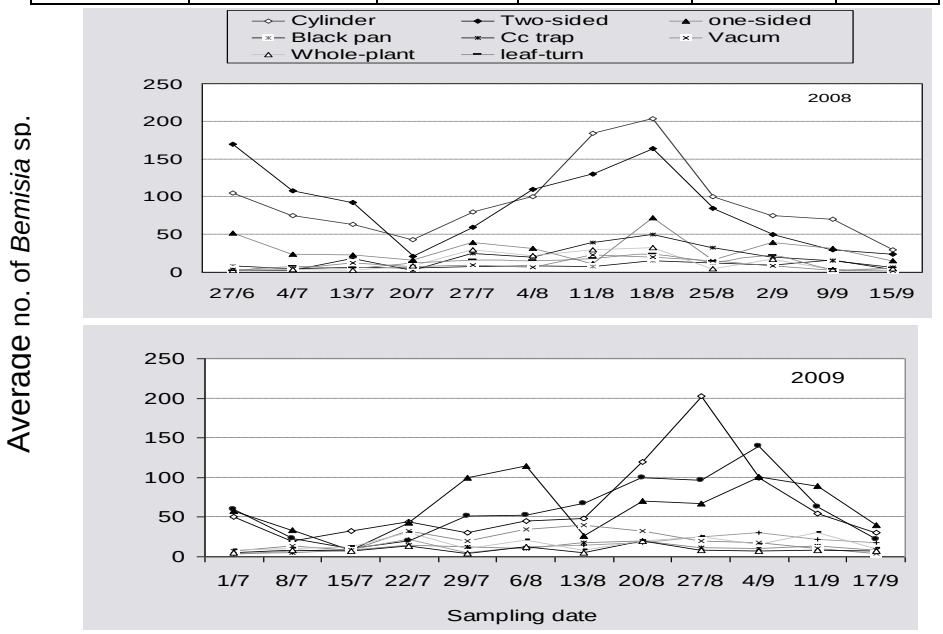

Figure (1): Relative population trends of *Bemisia* spp. estimated with cylinder sticky trap, two-sided sticky trap, one-sided sticky trap, CC trap, black pan, vacuum sampler, whole-plant, and leaf-turn on soybean plants during 2008 at Mansoura district.

Table (2) shows the mean number ±SEM of *Bemisia* spp., relative variation, sampling cost, and relative net precision for eight sampling techniques of *Bemisia* spp. The data indicated that the black pan had the lowest associated RV value in both seasons. Whereas, the whole-plant had the highest calculated RV value in the first season but in the second season the cylinder sticky trap had highest calculated RV value. The black pan

method had the highest calculated RNP value (most efficient) compared with all other sampling methods. Whereas, the vacuum sampler had lowest associated RNV value in both seasons. These findings disagree with the results of Naranjo and Flint (1995) who pointed out the leaf-turn method was the most reliable and efficient technique for estimating adult abundance of *Bemisia* spp. compared with black pan method and sticky traps.

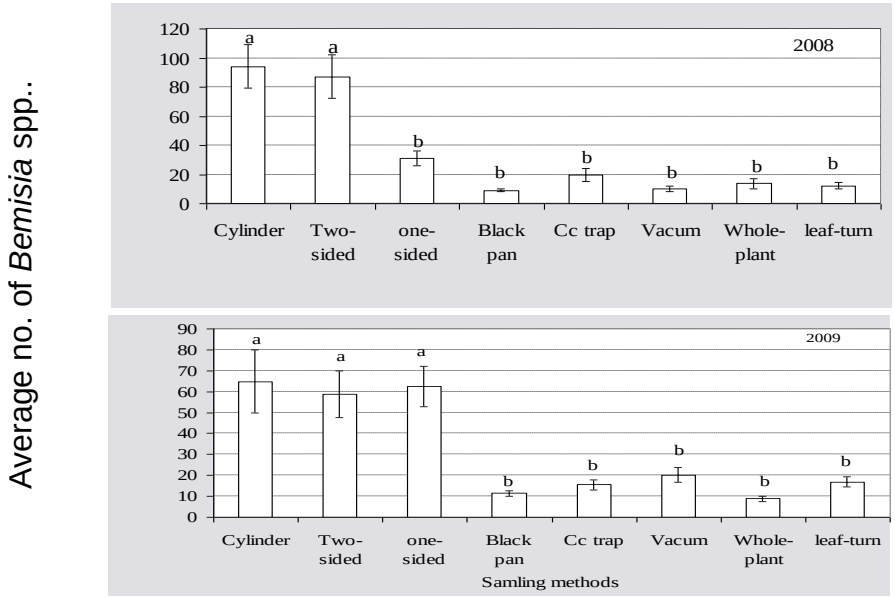

Figure (2): Mean number ± SE of adult *Bemisia* spp. collected with cylinder sticky trap, two-sided sticky trap, one-sided sticky trap, CC trap, black pan, vacuum sampler, whole-plant, and leaf-turn on soybean plants during 2008 and 2009 seasons at Mansoura district.

The average time required to collect and record the 12 sampling with each sampling methods is shown in Table (2). The black pan method and the whole-plant method required less time than the other methods. The vacuum sampler and leaf-turn required more time than the other methods.

The data presented in Figure (3) showed a comparison of percent of sampling methods capture of *Bemisia* spp. on soybean plants. Two-sided sticky traps recorded the highest percent of capture of *Bemisia* spp., then cylinder sticky traps during 2008 and 2009 seasons.

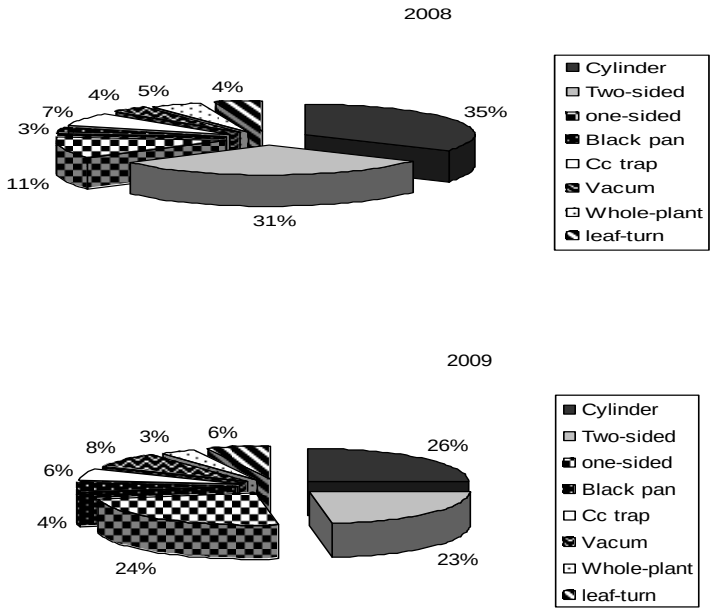

Figure (3): A comparison of percent of sampling methods capture to *Bemisia* spp. on soybean plants during 2008 and 2009 seasons at Mansoura district.

Table (2): Mean number ± SEM of *Bemisia* spp., relative variation (R.V.), sampling cost (C) and relative net precision (R.N.P.) for eight sampling technique of *Bemisia* spp. on soybean plants during 2008 and 2009 seasons at Mansoura district.

Sampling methods	Mean ±SEM	R.V	C	R.N.P.
2008				
Cylinder	94.16±15.0	15.9	0.13	48.5
Two-sided	87.08±14.9	17.1	0.13	45.04
One-sided	31.16±5.10	16.3	0.05	123.4
Black pan	9.16±1.10	11.9	0.02	434.3
Cc trap	19.75±4.30	21.8	0.04	114.4
Vacuum sampler	9.91±1.90	19.1	0.17	30.80
Whole-plant	13.75±3.40	24.8	0.02	204.1
Leaf turn	12.3±20	16.2	0.17	36.4
2009				
Cylinder	64.83±15.1	23.3	0.15	28.6
Two-sided	58.75±11.2	19.06	0.14	37.4
One-sided	62.58±9.7	15.5	0.08	80.6
Black pan	11.41±1.4	12.3	0.04	204.08
Cc trap	15.58±2.4	15.4	0.07	93.4
Vacuum sampler	20.16±3.5	17.4	0.20	28.73
Whole-plant	8.83±1.3	14.7	0.05	136.98
Leaf turn	16.83±2.4	14.3	0.21	33.3

Melanagromyza cunctans

As shown in Table (3), the count of *M. cunctans* was highly significantly effect by sampling methods. Figure (4) shows the weekly trap captures of *M. cunctans*. As influenced by sampling methods. All sampling methods indicated similar population trends throughout the first season. The numbers of insect were caught by all sampling methods are low on the first sampling date, and then increased during the growing season. The results indicated that the cylinder sticky traps were caught significant more adults of *M. cunctans*. Moreover, during the season of 2009, the numbers of insect were caught with whole-plant on the 1st week appeared with the highest numbers then decreased. Whereas, the number of insects caught with cylinder sticky traps was increased during the season. Scheirs *et al*. (1997) indicated that the agromyzid flies were trapped earlier in color traps compared to Malaise traps

There were significant differences between traps during 2008 and 2009 seasons as shown in Figure (5). Among the sampling methods, Cylinder sticky trap was caught the most adult of whitefly during the two seasons. Furthermore, there were no significant differences between yellow water pan traps, visual examination, and sweep-net. Moreover, there were no significant differences between one sided sticky, whole-plant, blue water pan, red water pan, and two-sided sticky traps. The percent of sampling methods capture to *M. cunctans* on soybean plants during 2008 and 2009 seasons are shown in Figure (6).

Table (4) shows the mean number of *M. cunctans* ± SE, relative variation, sampling cost, and relative net precision for nine sampling technique of *M. cunctans*. The data indicated that the cylinder sticky traps had the lowest associated RV value in 2008 season. Whereas, in 2009 season, two-sided sticky traps had lowest associated RV value. The blue water pan traps had the highest calculated RV value in 2008 and 2009 seasons. One sided sticky traps method had the highest calculated RNP value (most efficient) compared with all other sampling methods in season 2008 but in season 2009, two-sided sticky traps had the highest calculated RNP value (most efficient) compared with all other sampling methods. Whereas, the whole-plant had lowest associated RNP value in both seasons. Red, blue, and yellow water pan traps required less time than the other methods (Table 8).

Table (3): One way analysis of variance (ANOVA) for the impact of sampling method on the numbers of *M. cunctans* during 2008 and 2009 seasons at Mansoura district.

Factor	Sum of squares	Degrees of freedom	Mean square	F. Test	P
2008					
Method	1383.96	8	172.99	12.72	000***
Error	1345.9	99	13.595		
2009					
Method	1154.66	5	144.33	17.362	000***
Error	823	99	8.313		

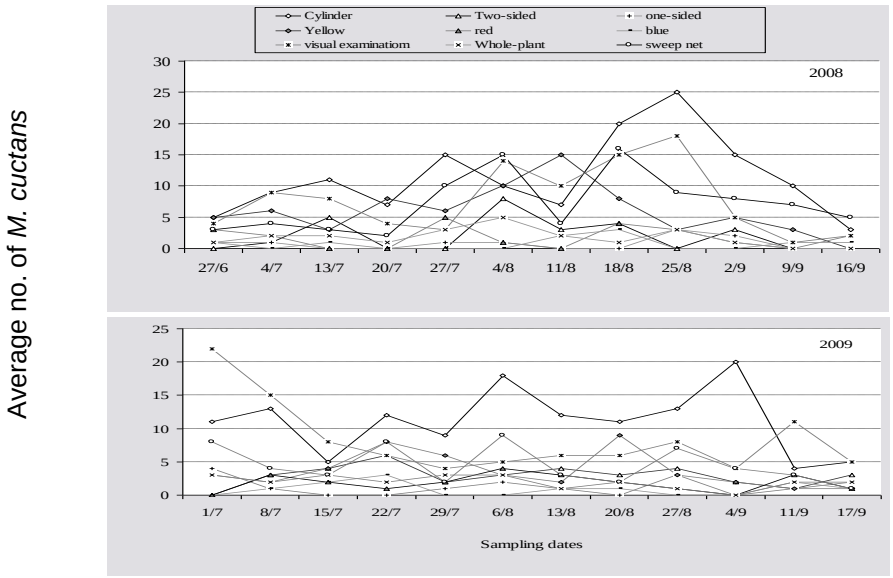

Figure (4): Relative population trends of *M. cunctans* estimated with cylinder sticky trap, two-sided sticky trap, one-sided sticky trap, whole-plant, yellow pan trap, red pan trap, blue pan trap and visual examination on soybean plants during 2008 and 2009 seasons at Mansoura district.

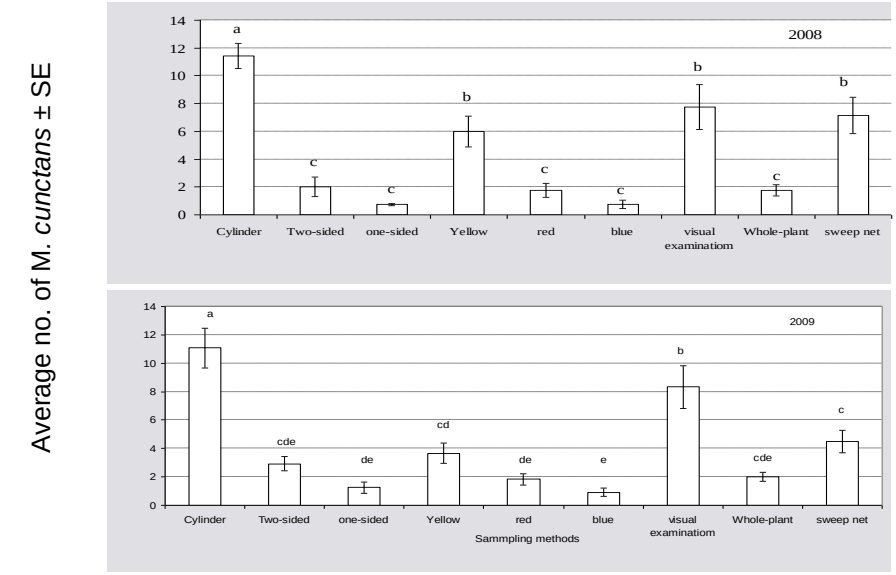

Fig. 5: Mean number ± SE of adult *M. cunctans* collected with cylinder sticky trap, two-sided sticky trap, one-sided sticky trap, whole-plant, yellow pan trap, red pan trap, blue pan trap, visual examination, and sweep-net on soybean plants during 2008 and 2009 seasons at Mansoura district.

Table (8): Mean number ± SE of *M. cunctans*, relative variation (R.V.), sampling cost (C), and relative net precision (R.N.P.) for nine sampling techniques on soybean plants during 2008 and 2009 seasons at Mansoura district.

Sampling methods	Mean ± SE	R.V	C	R.N.P
2008				
Cylinder	11.4±0.9	7.89	0.0040	3225.80
Two-sided	2±0.7	35.00	0.0040	714.20
One-sided	0.75±0.08	10.66	0.0027	3571.40
Yellow	6±1.12	18.66	0.0030	1785.7
Red	1.75±0.5	28.57	0.0030	1176.4
Blue	0.75±0.3	40.00	0.0030	833.33
Whole-plant	1.75±0.4	22.85	0.0080	549.4
Visual examination	7.75±1.6	20.64	0.0080	606.06
Sweep-net	7.16±1.3	18.15	0.0050	689.60
2009				
Cylinder	11.08±1.4	12.63	0.0050	1587.3
Two-sided	2.91±0.3	10.30	0.0050	1960.7
One-sided	1.25±0.4	32.00	0.0030	1041.6
Yellow	3.66±0.7	19.12	0.0035	1515.1
Red	1.83±0.4	21.85	0.0035	1315.7
Blue	0.91±0.3	32.96	0.0035	869.5
Whole-plant	2±0.3	15.00	0.0120	317.4
Visual examination	8.33±1.5	18.00	0.0120	492.6
Sweep-net	4.5±0.8	17.77	0.0080	704.2

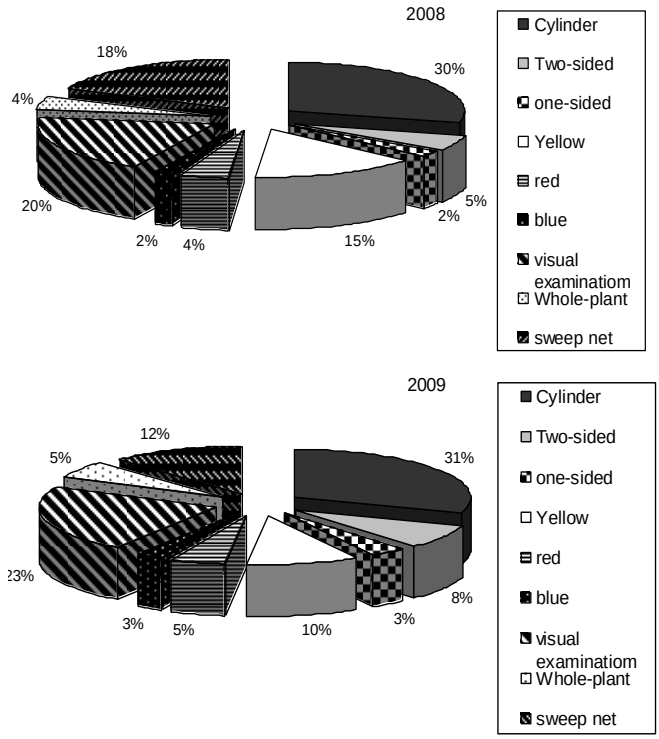

Fig. 6: A comparison of percent of sampling methods capture to *M. cunctans* on soybean plants during 2008 and 2009 seasons at Mansoura district.

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تقييم بعض طرق أخذ العينات لكل من الذباب الأبيض وذبابة ساق فول الصويا على نباتات فول الصويا فى منطقة المنصورة

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أجريت هذه الدراسة لتقييم بعض طرق أخذ العينات للحشرات الكاملة لكل من الذبابة البيضاء وذبابة ساق فول الصويا على نباتات فول الصويا خلال موسمى ٢٠٠٨ و٢٠٠٩ لتحديد مدى دقة وكفاءة هذه الطرق فى تحديد الحجم الحقيقى للتعداد فى الحقل. تم تقييم ثلاثة انواع من المصائد اللاصقة الصفراء (على شكل اسطوانى ، المصائد ذات الوجهين اللاصقين، وذات الوجه الواحد اللاصق) ، ثلاثة ألوان مختلفة للمصائد المائية، CC trap ، شبكة الجمع كطرق للعدّ الغير مباشرة التى تعتمد على نشاط الحشرة والفحص المباشر والوعاء الأسود كطرق مباشرة للعدّ التى تعتمد على التواجد الفعلى على النبات.

أوضحت النتائج ان المصائد الصفراء على شكل اسطوانى أكثر الطرق جمعاً للذبابة البيضاء. أشارت النتائج إلى عدم وجود معنوية بين المصائد ذات الوجهة الواحد اللاصق CC trap, Black pan, Vacuum sampler, Whole-plant والفحص المباشر خلال الموسم الأول. بينما سجلت طريقة Black pan اقل قيمة للإختلاف المطلق خلال موسمى الدراسة ، بينما سجلت طريقة Whole-plant

أعلى قيمة للإختلاف المطلق فى الموسم الأول بينما فى العام الثانى سجلت المصائد الصفراء على شكل اسطوانى أعلى قيمة .أما طريقة Black pan سجلت أعلى قيمة لقيمة الدقة النسبية مما يدل على أنها اكثر الطرق كفاءة لتقدير الحجم الحقيقى للتعداد للذبابة البيضاء.

أظهرت النتائج ايضا وجود فروق معنوية بين طرق اخذ العينات لذبابة ساق فول الصويا حيث سجلت المصائد الصفراء على شكل إسطوانى أعلى تعداد لذبابة ساق فول الصويا خلال موسمى الدراسة كذلك عدم وجود معنوية بين المصائد المائية الصفراء ، شبكة الجمع والفحص المباشر خلال الموسم الأول بينما فى العام الثانى أوضحت النتائج عدم وجود معنوية بين المصائد ذات الوجهة الواحد اللاصق ، Whole-plant ، المصائد المائية الزرقاء ، شبكة الجمع والفحص المباشر المصائد المائية الحمراء والمصائد ذات الوجهين اللاصقين . بينما سجلت طريقة المصائد الصفراء على شكل إسطوانى أقل قيمة للإختلاف المطلق خلال الموسم الأول ، بينما سجلت طريقة المصائد ذات الوجهين اللاصقين أقل قيمة للإختلاف المطلق فى الموسم الثانى . كما سجلت المصائد ذات الوجه الواحد اللاصق أعلى قيمة لقيمة الدقة النسبية مما يدل على أنها اكثر الطرق كفاءة لتقديرالحجم الحقيقى للتعداد لحشرة ذبابة ساق فول الصويا فى الموسم الأول بينما فالموسم الثانى سجلت المصائد ذات الوجهين اللاصقين أعلى قيمة لقيمة الدقة النسبية.

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