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Evaluation of bio pesticidal formulations of plant origin against *Cyllodes indicus* (Coleoptera: Nitidulidae) in *Pleurotus sajor caju* (*In vivo*)

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Abstract
Three biopesticidal formulations of plant origin viz., Max Raze, Max Cannon and Neemazal when tried against *C. indicus* showed high efficacy for first fortnight after application as evident from statistically similar mushroom production during this period in the bags receiving insects and formulations and insect free bags in *Pleurotus sajor caju*. However, due to the speculated low persistence of these formulations, the insect population started building up after about twenty days of application, thus reducing the yields in third and fourth week of cropping. However, significantly improved average yields were recorded in biopesticides receiving treatments as compared to the bags in which only adults of *C. indicus* were released. Among the tested formulations, Max Raze yielded best results followed by Max Cannon. The least effective was Neemazal, though, yield improvement by this formulation was also significant. Visually, no infestation of sporocarps was observed during first and second week, but with the reducing efficiency of biopesticides, more and more sporocarps got infested. Highest sporocarp infestation among the treated bags was observed in Neemazal treated bags.

Keywords: *Cyllodes indicus*, *Pleurotus sajor caju*, biopesticides, sporocarps

Introduction

Among the commercial cultivated mushrooms, Oyster mushrooms (*Pleurotus* spp.) with contribution of 24.1 per cent of the total world production rank third, next only to White Button Mushrooms (*Agaricus* spp.) and Shitake (*Lentinula edodes*). The factors that go in favour of this mushroom are its desirable traits like scrumptious taste, unique flavor, high nutritional status, medicinal values, biotechnological applications and overall, its simple and economical cultivation techniques that enables even an uneducated farmer to grow it after a training of two to three days (Balakrishan and Nair, 1997; Cohen *et al.*, 2002, Yildiz and Yesil, 2006 and Khare *et al.*, 2007) [5, 14, 9]. But its pace of growth is slow in India due to the incidence of many insect pests belonging to order Diptera and Coleoptera that has been recorded during its cultivation (Kumar and Sharma, 2001; Cline and Leschen, 2005 and Kumar, 2006) [2, 4]. Of various coleopteran insect pests which effect the cultivated oyster mushrooms various species of *Cyllodes* viz., *Cyllodes bipligiatus, C. ater* and *C. literatus*, *C. whiteii*, *C. bifacies* and *C. indicus* (Boving and Rozen, 1962; Hayashi, 1978; Johal *et al.*, 1992; Gnaneswaran and Wijayagunasekara, 1996 and Sharma, 2010) [3, 7, 8, 6] have been reported from oyster mushroom farms of different regions of the world including Himachal Pradesh. Neem formulations like Rakshak and Neemark proved highly effective against coleopteran insect pests (Bhat *et al.*, 1998) [2]. Achook (azadirachtin 0.15% EC) and Nimbecidine (azadirachtin 0.03% EC) also showed effective coleopteran beetle control in Oyster mushrooms with lowest fruit body infestation of crops (Mazumder *et al.*, 2005) [12]. As, *C. indicus*, with only recent records of occurrence in Himachal Pradesh showed increase in its frequency of distribution with every growing season in the farms growing *Pleurotus* spp. indicating it to acquire the status of a pest if not checked in time. A strategy was thus planned to reduce its infestation by using different crop friendly formulations of plant origin with known insecticidal properties viz., Max Raze [*Ocimum sanctum* L. (10%), *Andropogon nardus* L. (30%), Samesol (25%) and Nerolidol (20%)], Max Cannon (10, 000 ppm of azadirachtin) and Neemazal [azadirachtin (1%) and neem oil (46.3%)] have been evaluated for the management of *C. indicus* in oyster mushroom farms.

Materials and Methods

Polypropylene bags containing three kg substrate medium were sprayed separately with three biopesticidal formulations viz., Max Raze @ 0.1 %, Max Cannon @ 0.1 % and Neemazal @ 0.005 % two days prior to pin head formation to test their efficacy against *C indicus*. The adults of *C indicus* were released @ 20 individuals per bag at the time of pin head formation stage. Untreated insect free bags were maintained as control I and insect released but untreated bags were maintained as control II. Each treatment was replicated three times and data were analyzed through CRD.

Sporophore yield on day to day basis was taken for each replication separately for all the treatments as well as two controls from day one of sporocarp appearance to the end of cropping. Sporocarp yields were assimilated at weekly intervals to calculate the total sporocarp production. All the sporocarps as and when harvested were observed for infestation by grubs and/ or adults. Per cent infestation was worked out in relation to total sporocarp yield. The populations of test insect (grubs and adults) per 50 g of sporocarps were assessed in 3rd as well as 4th weeks in all the insect released treatments.

Resuts and Discussion

Experiment conducted to test the efficacy of plant formulations viz., Max Raze, Max Cannon and Neemazal against *C. indicus* which eventually influenced the mushroom yields revealed no phytotoxicity on *P. sajor caju* with the tested doses of referred biopesticides.

Table 1: Effect of plant biopesticides on weekly sporocarp production of *P. sajor caju* in presence of *C. indicus*

Treatments	*Mean sporocarp yield (g) in the indicated weeks				Mean
	first week	second week	third week	fourth week	
Max Raze @ 0.1 % + 20 adults	726.6 (2.86)	390.0 (2.59)	146.0 (2.16)	75.0 (1.87)	334.4 (2.37)
Max Cannon @ 0.1 % + 20 adults	713.3 (2.85)	356.7 (2.55)	98.3 (1.99)	53.3 (1.71)	305.4 (2.28)
Neemazal @ 0.005 % + 20 adults	600.0 (2.78)	330.0 (2.52)	48.3 (1.67)	5.0 (1.17)	245.8 (2.04)
Untreated control + 20 adults	390.0 (2.58)	160.0 (2.18)	22.0 (1.33)	0.0 (0.0)	143.0 (2.03)
Untreated insect free control	718.3 (2.85)	401.7 (2.60)	336.7 (2.52)	161.7 (2.20)	404.6 (2.54)
Mean	629.7 (2.78)	327.7 (2.49)	130.2 (1.94)	59.0 (1.74)	

*Average of three replications

Figure in parentheses are log transformed values

$CD_{0.05}$		
	Treatments (T)	0.08
	Weeks (W)	0.07
	T x W	0.15

Neemazal was found to be least effective producing mean sporocarp yield of 245.8 g which was significantly similar to 143.0 g obtained in insect released untreated control. Max Raze and Max Cannon, though effective, could not produce yields to the level of insect free bags. The mean sporocarp production of 334.4 g produced in Max Raze treated bags was significantly lower than 404.6 g produced in untreated insect free bags but significantly more than 305.4 g produced in Max Cannon treated bags. Mean sporocarp production declined significantly at weekly intervals. Highest mean sporocarp production of 629.7 g during first week was significantly higher than 327.7 g produced during second week. The production declined further to 130.2 g during third week and nearly 59.0 g were produced in fourth week. Weekly mushroom production in various treatments produced some interesting data, wherein, sporocarp yields during first week and second week in all the biopesticides treated bags were at par with untreated insect free bags. However, whereas in untreated insect free control bags, mushroom production of 336.7 g produced in third week was at par with that of 401.7 g produced during second week, it declined significantly in all the bags receiving biopesticides formulations. Among the formulation receiving treatments, higher yield of 146.0 g was obtained in Max Raze treated bags during third week which was significantly more than 98.30 g produced in Max Cannon treated bags. Minimum and significant lower yield of 48.3 g was produced in treatment receiving Neemazal. Progressive and significant decline was recorded from third to fourth week in all the treatments including control. The information gathered from this data indicated overall better efficacy of Max Raze and Max cannon as compared to Neemazal. All the formulations showed low persistence as their impact was felt on the yields only for two weeks. Thereafter, insect population started showing up that declined the yield significantly as compared to untreated insect free bags (Table 1).

Observations regarding efficiency of these plant formulations against *C. indicus* affecting sporocarp production of oyster mushrooms placed in Table 2 showed highest mushroom production of 1618.3 g in untreated insect free control bags which was statistically higher than the yield of 1338.0 g obtained in bags that received apparently best looking formulation of Max Raze. Sporocarp production in the bags treated with Max Raze and Max Cannon was significantly at par with each other. Neemazal treated bags produced 983.3 g fruiting bodies, which though, statistically significantly lower than the yields obtained in bags treated with Max Raze and Max Cannon, were significantly higher than the yields of 572.0 g produced in untreated insect released control. Per cent yield losses to the tune of 17.3, 24.5 and 39.3 were assessed in respective treatments of Max Raze, Max Cannon and Neemazal. An appreciably high yield loss of 64.7 per cent was recorded in untreated but insect released control bags. The information gathered from this table signifies the efficiency of tested biopesticidal formulations against *C. indicus* especially Max Raze and Max Cannon. Looking into the insect pest scenario of mushrooms and least possibility of use of synthetic chemicals in the closed biological cropping system with fresh consumption of fruiting bodies, the results delivered by these plant formulations carry a great significance and need to be exploited against pests other than *C. indicus* also. Their low persistence can be taken care of with their repeat application after three weeks of first one.

Table 2: Effect of plant biopesticides on total sporocarp yield of *P. sajor caju*

Treatments	*Total yield per bag (g)	Per cent yield loss
Max Raze @ 0.1 % + 20 adults	1338.0	17.3
Max Cannon @ 0.1 % + 20 adults	1222.0	24.5
Neemazal @ 0.005 % + 20 adults	983.3	39.3
Untreated control + 20 adults	572.0	64.7
Untreated insect free control	1618.3	
$CD_{(0.05)}$	186.4	

*Average of three replications

There were no apparent symptoms of infestation on the sporocarps during first and second weeks of cropping in any of the treatments except untreated insect released control which were infested to the tune of 52.69 and 83.86 per cent. However, symptoms of infestation became apparent during third week in all the treatments in form of presence of grubs and/or adults of *C. indicus*. The data have been analyzed statistically through analysis of Factorial CRD technique (Table 3).

Observations taken third week onwards showed significantly progressive infestation with progression of time. Mean sporocarp infestation of 76.68 per cent recorded during third week reached the level of 96.52 per cent during fourth week. Bags treated with Max Raze showed 65.57 per cent mean infested sporocarps which were significantly lower as compared to 82.20 per cent infestation observed in Max Cannon treated bags. Best results were obtained in the bags treated with Max Raze in which only 39.01 per cent of the total sporocarps produced during third week showed infestation which was significantly lower than any of the other treatments during this period. Poorest results were achieved with Neemazal in which 96.06 per cent of the total sporocarps produced in third week were infested. Cent per cent sporocarps were found to be infested in the produce of fourth week. While a significant difference in the per cent infestation in the treatments receiving Max Raze and Max Cannon was recorded during third week, the infestation during fourth week in these treatments (92.13 and 93.94 per cent respectively) was statistically at par with each other. Among various biopesticide treatments, Neemazal seemed to be the least effective and Max Raze, the most effective. This pattern of no sporocarp infestation by *C. indicus* during first fortnight and progressively increased infestation thereafter, supported that probably the tested biopesticides had a low persistence and as they lost their persistence, more and more number of sporocarps got infested by the insect.

Table 3: Efficacy of biopesticides against *C. indicus* in terms of per cent infestation of *P. sajor caju* sporocarps

Treatments	*Infestation in the indicated weeks (%)		Mean
	Third week	Fourth week	
Max Raze @ 0.1 % + 20 adults	39.01 (38.65)	92.13 (73.70)	65.57 (54.07)
Max Cannon @ 0.1% + 20 adults	71.67 (57.84)	93.94 (75.75)	82.20 (65.04)
Neemazal @ 0.005% + 20 adults	96.06 (78.55)	100.0 (90.00)	98.03 (81.93)
Untreated control + 20 adults	100.0 (90.00)	100.0 (90.00)	100.0 (90.00)
Mean	76.68 (61.12)	96.52 (79.24)	

*Average of three replications
Figure in parentheses are arc sine transformed values

$CD_{0.05}$		
	Treatments (T)	7.30
	Weeks (W)	5.17
	T x W	10.34

As the adults were released in the bags treated with referred biopesticides as well as untreated control, a few adults were found sluggish/knock down/dead within two to three days of release in the bags treated with biopesticides. However, the tested formulations seemed to have some repellent/antifeedent action as more number of adults was found to feed on the mycelium/emerging pins in the control bags in the initial phase (first week) of release. The surviving individuals multiplied and their population reached the peak during third week of cropping. The data of grub and adult population in 50g of sporocarps during third and fourth week have been presented in Table 4. Visual observations during first week of cropping revealed the presence of beetles in biopesticides free bags only. However, with progression of time, the population started showing up in all the treatments. Minimum mean count of 30.08 insects recorded in Max Raze treated bags during third and fourth weeks was statistically lower than all other treatments. Mean insect population of 62.91 and 73.50 recovered in Max Cannon and Neemazal treated bags were statistically at par with 83.58 insects retrieved in control bags. Maximum mean grub population of 173.5 was observed during third week which declined significantly to 43.50 during fourth week. Mean adult population of 28.75 counted during third week was also significantly higher than 4.33 adults present during fourth week. Grub as well as adult populations were at its peak during third week and started declining during fourth week of observation. While the decline in grub population from third to fourth week was not significant in Max Raze and Max cannon treated bags, it was highly significant in Neemazal and untreated insect receiving control bags. Also, the adult population of 17.00 insects recovered during third week in Max cannon treated bags declined significantly to just 3.33 in fourth week.

Presumably, the adults due to their flying behavior had a tendency to avoid the mycelium of bags treated with biopesticidal formulations in the initial phases of treatments, which had some repellent/antifeedent action. As the persistence of formulations reduced with time, the adults shifted to treated bags and infested the mycelium/sporocarps and multiplied as evident from higher grub population observed during third and fourth weeks.

Table 4: Effect of plant biopesticides on multiplication of *C. indicus* (recorded in 50 g of sporocarps)

Treatments	*Population in third week		*Population in fourth week		Mean
	Grubs	Adults	Grubs	Adults	
Max Raze @ 0.1 % + 20 adults	59.33 (1.76)	8.67 (0.92)	46.67 (1.67)	5.67 (0.74)	30.08 (1.47)
Max Cannon @ 0.1% + 20 adults	130.67 (2.11)	17.00 (1.21)	100.67 (2.0)	3.33 (0.52)	62.91 (1.79)
Neemazal @ 0.005 % + 20 adults	223.00 (2.35)	36.00 (1.56)	26.67 (0.63)	8.33 (0.46)	73.50 (1.86)
Untreated control + 20 adults	281.00 (2.45)	53.33 (1.73)	0.0 (0.0)	0.0 (0.0)	83.58 (1.92)
Mean	173.50 (2.16)	28.75 (1.35)	43.50 (1.07)	4.33 (0.63)	

*Average of three replications

Figure in parentheses are log transformed values

$CD_{0.05}$		
	Treatments (T)	0.20
	Population (P)	0.23
	T x P	0.40

Conclusion

Three botanical biopesticidal formulations viz., Max Raze, Max Cannon and Neemazal, when tested against *C. indicus* showed high efficacy for the first fifteen days which resulted into improved mushroom production during first two weeks. However, no effect of these biopesticides was observed beyond this period, when the surviving insect fed upon the crop, multiplied freely and resulted into appreciable yield losses. Max Raze yielded best results in form of highest crop yields followed by Max Cannon and Neemazal was found to be the least effective, among the three.

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